Development of ozonation and ceramic membrane filtration combination process for water reclamation considering virus removal and disinfection by-product formation

2017

DONGBUM IM

Development of ozonation and ceramic membrane filtration combination process for water reclamation considering virus removal and disinfection by-product formation

（ウイルス除去と消毒副生成物の生成を考慮した
オゾンとセラミック膜ろ過の組合せ
下水再生プロセスの開発）

DONGBUM IM

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the requirements for the degree of
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Department of Urban and Environmental Engineering
Graduate School of Engineering
Kyoto University
Kyoto, Japan

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Chapter Ⅰ

Introduction

1.1 Research background

Water is one of the most essential natural resources for human. However, the water scarcity problem has been made worse due to increasing water demand and diminishing water resources caused by global population growth, urbanization and climate change since the Industrial Revolution. According to recent researches, global water demand has tripled since the 1950s (Gleik, 2003). Moreover, a number of people who live in water stressed or water scarce contries will grow to three billion (Molden et al., 2007). At the same time, the limited freshwater resources in rivers, lakes and groundwater aquifers are dwindling due to over-exploitation and water quality degradation (Tilman et al., 2002). Under these circumstances, the necessity of water reclamation has been emphasized in order to cope with the water scarcity problem.

Wastewater, which has the consistent quantity and quality even under droughts and other climatic conditions, has been received attetion as alternative water resources. It is expected to derive numerous benefits from wastewater reclamation. For example, wastewater recycling contribute to prevention of aquatic environment pollution through the reduction of the volume of discharge water from wastewater treatment plant (Asano et al., 2006). It can also save the energy consumption for water supply caused by diminishing the volume of water intake. On the other hand, wastewater contains diverse contaminants including pathogens, chemicals and other toxins. The public health of reclamid water users will be threaten if these contaminants were not eliminated adequately during water treatment. Moreover, water treatment process required

enormous energy consumption to eliminate diverse contaminants and it is another problem which water reclamation was faced with. Thus, it is needed to develop the efficient treatment process in order to provide hygienically safe reclaimed water.

In this study, among the many treatment technologies, ozonation and ceramic membrane filtration combination process (O_3&CMF process) was selected as a treatment process for water reclamation. By incorporating ozonation with cermic membrane filatrion, it was expected that membrane fouling, which causes a performance deterioration of membrane, can be mitigated (Kim et al., 2008; Park et al., 2010; Van Geluwe et al.,2011; Zhang et al., 2013; Fan et al., 2014; Cheng et al., 2016; Wei et al., 2016). Also, It has been well documented that ozonation could inactivate virus effectively (Kim et al., 1980; Roy et al., 1981; Tyrrell et al., 1995; Shin et al., 2003; Fang et al., 2014). However, there is a possibility to be produced disinfection by-products (DBPs) which has been known as human carcinogens during ozonation (Glaze et al., 1987; Schechter et al., 1995; Gagnon et al., 1997; Kuo et al., 1998; Richardson et al., 1998; Can&Gurol, 2003). Thus, it is necessary to evaluate comprehensively both operational and treatment performance.

From this reason, this study aims to develop efficient O_3&CMF process considering the protection of public health.

1.2 Research objective

According to the above research background, detailed objectives of this study are as follows:

1. To evaluate operational performance of O_3&CMF process through long term operation
2. To investigate the formation of disinfection by-products and virus removal performance
3. To investigate the applicability of reclaimed water based on risk assessment considering virus removal and disinfection by-products formation
4. To determine a novel monitoring indicator in order to ensure a reliability of O_3&CMF process and to maintain full protection of public health

1.3 Research structures

This dissertation consists of nine chapters. As can be seen in Figure 1.1, the structure of this research work is described with a general outline of each chapter.

In Chapter I, research background, objectives and structure was described. A literature review was summarized in Chpater II.

In Chapter III, the virus removal performance of both ozonation and coagulation was evaluated through lab scale experiment, and also the effect of pretreatment on subsequent treatment was investigated. On the basis of these performance evaluation and the assessment of energy consumption, the efficient process sequence in accordance with source water was selected prior to the continuous operation of O_3&CMF process.

In Chapter IV, the operational performance was evaluated through long term continuous operation with CEB, and also virus removal performance was evaluated using bacteriophage MS2 (MS2) as a model virus. On the basis of the result of performance evaluation, energy consumption was calculated, and also the applicability of reclaimed water produced by O_3&CMF process was suggested.

In Chapter V, both DBPs formations in O_3&CMF process and the formation potentials by chlorine disinfection was investigated. In addition, the effect on not only the removal of DBPs but also ceramic membrane filtration caused by adding BAC to O_3&CMF process were investigated.

In Chapter VI, risk assessment of reclaimed water produced by O_3&CMF process was conducted considering virus and DBPs based on the the result of Chapter IV and V.

In Chapter VII,the removal of both indigenous virus and FPH in wastewater by O_3&CMF process were investigated. Furthermore, the removal of each genotype of infectious FPH was evaluated through quantitative genotyping using IC-RT-PCR assays. In addition, the obtained results were compared with that of MS2 spike test to investigate a difference between the removal performance of O_3&CMF process on indigenous viruses and MS2 artificially spiked.

In Chapter VIII, a novel water treatment monitoring systems, which make it possible to take action instantly when treatment fails, would be determined to develop O_3&CMF process which consistently provide hygienic safety reclaimed water.

Finally, conclusions from this research and recommendations for further study were summarized in Chapter IX.

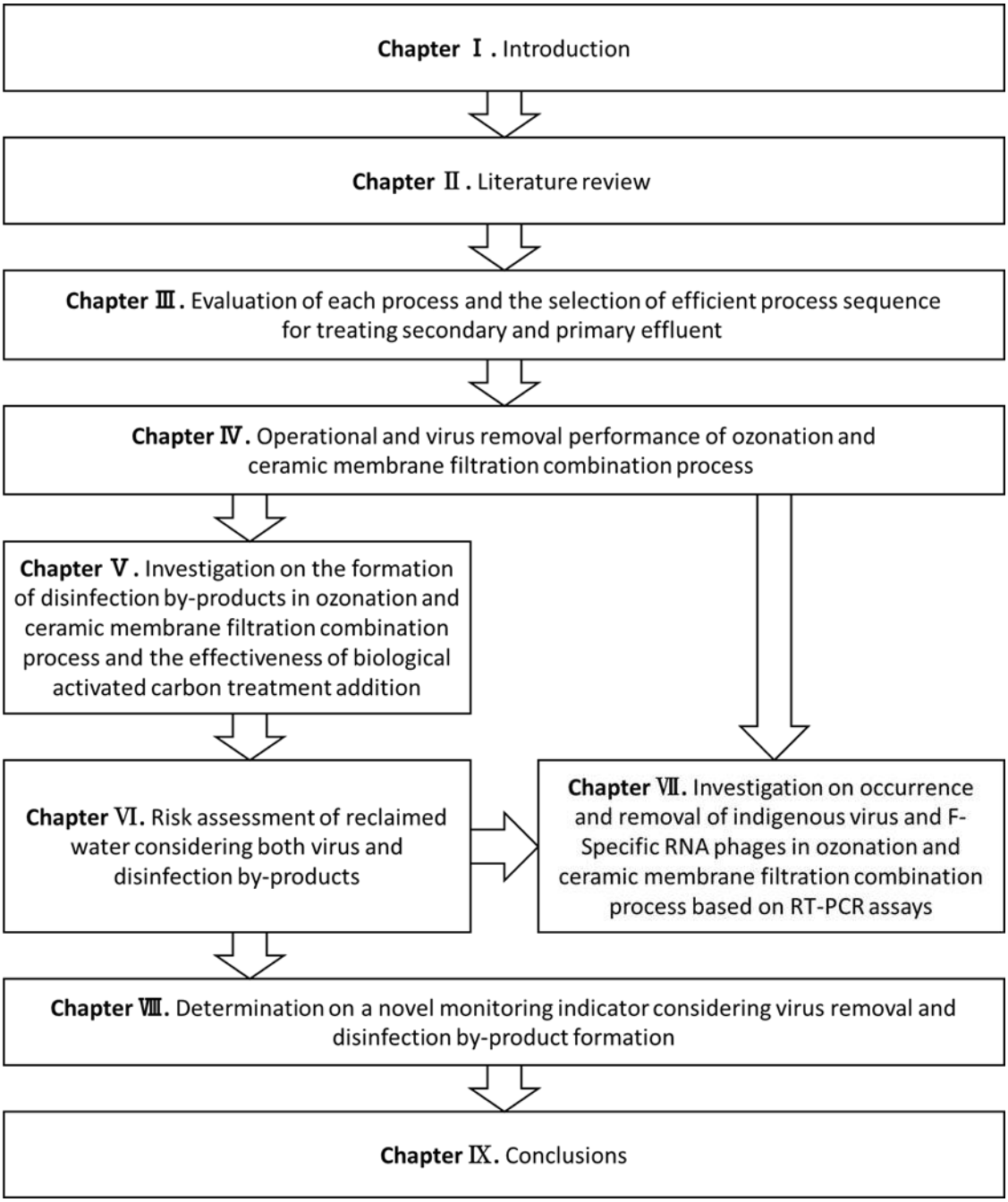

Figure 1.1 Schematic diagram of research structure

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Chapter Ⅱ

Literature Review

2.1 Circumstances of water reclamation

2.1.1 Japan

According to a reference announced by the Ministry of Land, infrastructure, Transport and Tourism (MLITT) in 2014, 187 million m^3 of wastewater was reused in 2011. Only 1.26% of wastewater was reused although 14.8 billion m^3 of wastewater was produced annually. Approximately 75% of reclaimed water was used for landscape irrigation, river flow maintenance and snow melting (Figure 2.1).

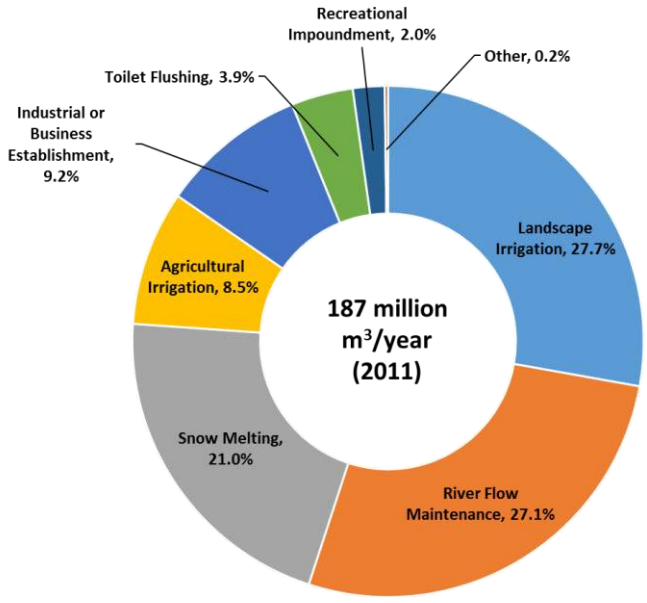
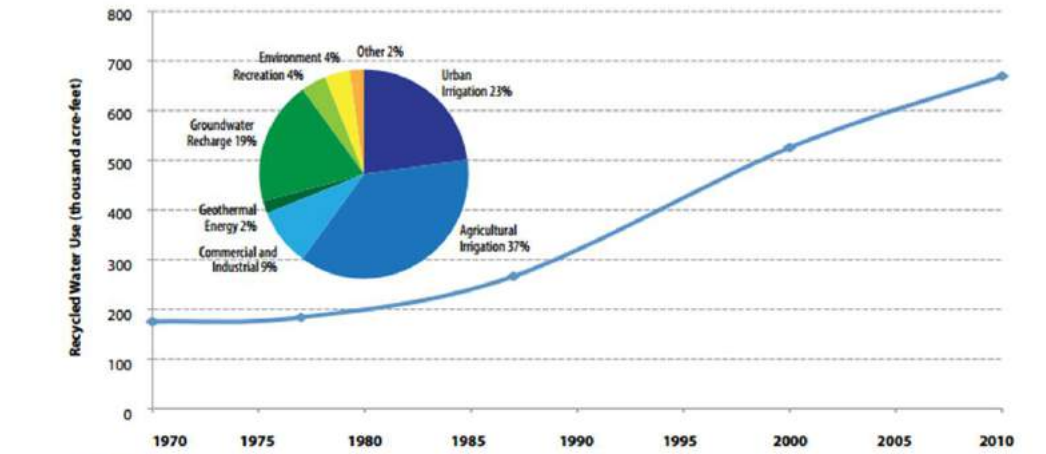


Figure 2.1 Reclaimed water use in Japan (MLITT, 2014)

2.1.2 America

Although there are no federal regulations directly governing water reuse practices in America, water reuse regulations have been developed by many individual states. Among these states, water has been reusing for more than 100 years in California. The earliest reclaimed water survey, conducted in 1970, found that an estimated 216 million m^3 of municipal wastewater was reused annually, about two-thirds of which was for agriculture (SWRCB, 1990). The volume of reclaimed water increased gradually, and 862 million m^3 of municipal wastewater was reused in 2009 (SWRCB and DWR, 2012). This accounts for 13% of the 6.17 billion m^3 of municipal wastewater produced annually in California. 37% of reclaimed water was used for agriculture. 17%, 12% of them was used for landscape irrigation, groundwater recharge, respectively (Figure 2.2).



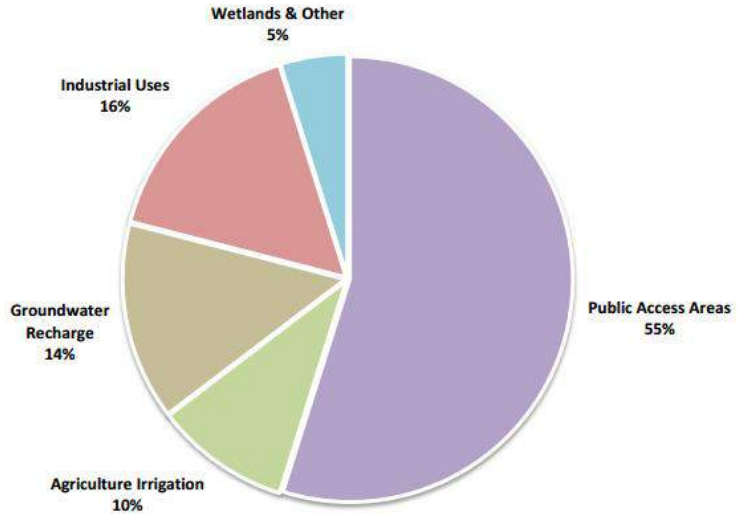
Source: SWRCB and DWR (2012)

Note: Urban irrigation includes the use of recycled water for irrigating large landscapes and golf courses. Groundwater recharge includes the use of recycled water for that purpose and as a seawater intrusion barrier.

Figure 2.2 Reclaimed water trends in California, 1970-2009, and (inset) reclaimed water use in 2009 (SWRCB and DWR, 2012)

Florida is also one of a leading state with respect to water reuse. Reclaimed water has been using since the 1960s with purposes for agricultural in Florida. According to a result of survey announced by Florida Department of Environmental Protection (FDEP), approximately 1 billion m^3 of reclaimed water was used for various purposes in 2014. This accounts for 44% of the total wastewater produced annually in Florida. Around 55% of reclaimed water was used for public access area and landscape irrigation, such as golf course or residential irrigation (Figure 2.3). 16% of reclaimed water was used for industrial, and 14%, 10% was used for groundwater recharge, agriculture irrigation,

respectively.



Note: (1) Agriculture irrigation includes edible crops (e.g., citrus) as well as feed and fodder crops (e.g., sprayfields).

Figure 2.3 Reclaimed water use in Florida (FDEP, 2014)

2.1.3 Korea

Water reclamation has been increase gradually from 174 million m^3 in 2001 to 678 million m^3 in 2008 in Korea. According to recent sewer statistics announced by the Ministry of Environment (ME) in 2014, 942 million m^3 of wastewater was reused in 2014 (ME, 2014). It accounts for 13.5% of wastewater (6.99 billion m^3) produced in 2014. 52.9% of reclaimed water was used at inside wastewater treatment plant (WWTP) with the purpose of cleaning, cooling and etc., and 47.1% (444 million m^3) was used at outside (left graph in Figure 2.4). The main purpose of reclaimed water used at outside WWTP is river flow maintenance (right graph in Figure 2.4). Although the uses of reclaimed water is limited, it was found that the reuse rate of wastewater is higher than that of Japan.

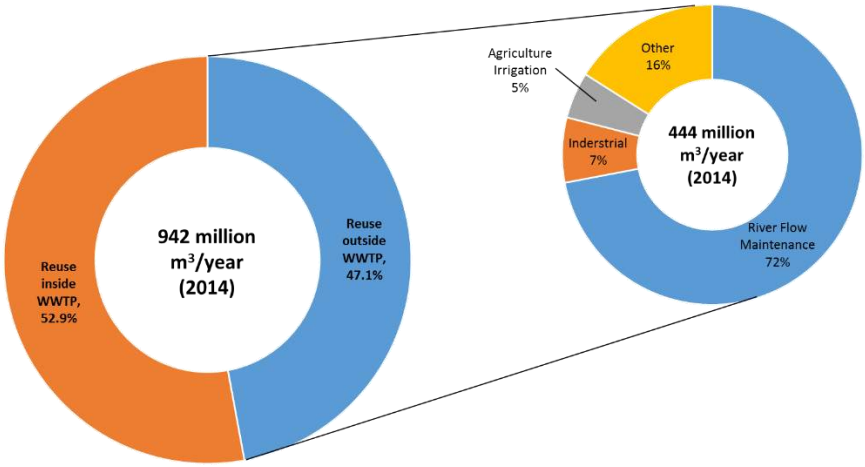


Figure 2.4 Reclaimed water use in Korea (ME, 2014)

2.1.4 Water reuse Regulation

Guidelines for Water Reuse has been updated in 1992, 2004 and 2012 since it was first published by U.S. Environmental Protection Agency (U.S. EPA) in 1980. New application, advances in technologies and also regulatory information related with water reuse were updated (U.S. EPA, 1992, 2004, 2012). Current status of regulations in 44 states was covered in this guidelines. If reclaimed water was used for applications where no direct public contact with the water, the guidelines recommend that a fecal coliform should be disinfected to achieve the concentration below than 200 Colony Forming Unit (CFU) /100mL. In case of uses where direct or indirect contact with reclaimed water is expected, no detectable fecal coliform per 100 mL is recommended as a minimum treatment goal. In order to meet this disinfection objective, filtration is generally required. On the other hands, there are no suggestion related with virus in this guidelines because a significant information exists indicating that the enteroviruses are reduced or inactivated via appropriate wastewater treatment.

California Department of Public Health (CDPH), formerly Department of Health and Safety, has established regulations covering reclaimed water named “Title 22”. Full treatment, which consists of cogulation, filtration and disinfection with ozonation or chlorine, is required in case of uses where direct or indirect contact with reclaimed water is expected, such as school yards, residential landscaping and parks. In this case, Title 22 restricts the median concentration of total coliform bacteria to be less than 2.2 Most Probable number (MPN)/100mL. Moreover, a disinfection process, when combined with the filtration process, should demonstrate that it is able to inactivate or remove 99.999%

(5log) of plaque forming units of F-specific bacteriophage MS2, or polio virus in wastewater. A virus at least as resistant to disinfection as polio virus may be used for purposes of demonstration.

Table 2.1 Allowable uses of reclaimed water (State of California, 2001)

Reclaimed water	USES
Disinfected Tertiary Recycled Water	1. Food crops, including all edible root crops where the edible portion comes into contact with the edible portion of the crop 2. Parks and Playgrounds 3. School yards 4. Residential landscaping 5. Unrestricted access golf courses 6. Any other irrigation use not specified in this section and not prohibited by other sections of the California Code 7. Nonrestricted recreational impoundment's
Disinfected Secondary Recycled Water (Max 2.2 MPN Coliform Bacteria per 100 Milliliters of Sample)	1. Food crops where the edible portion is produced above ground and not contacted by the recycled water 2. Restricted recreational impoundment's
Disinfected Secondary Recycled Water (Max 23 MPN Coliform Bacteria per 100 Milliliters of Sample)	1. Cemeteries 2. Freeway landscaping 3. Restricted access golf courses 4. Ornamental nursery stock and sod farm where access by the general public is not restricted 5. Pasture for animals producing milk for human consumption 6. Any non-edible vegetation where access is controlled so that irrigated areas can not be used as if it were part of a park, playground or schoolyard
Undisinfected	1. Orchards where recycled water does not come into contact with the edible portion of the crop 2. Vineyards where the recycled water does not come in contact with the edible portion of the crop 3. Non food bearing trees (Christmas tree farms are included in this category provided no irrigation with recycled water occurs for a period of 14 days prior to harvesting or allowing access by the general public 4. Fodder and fiber crop and pasture for animals not producing milk for human consumption 5. Seed crop not eaten by humans 6. Food crops that must undergo commercial pathogen destroying processing before being consumed by humans 7. Ornamental nursery stock and sod farms provided no irrigation with recycled water occurs for a period of 14 days prior to harvesting, retail sale, or allowing access by the general public

In Japan, Manual for Water Reclamation and Reclaimed water Quality Standard was established by MLITT in 2005. This guideline suggests water qualities depending on the four broad uses of reclaimed water (toilet flushing, sprinkling water, landscape irrigation and recreational impoundment). Table 2.2 shows the water reclamation guidelines in Japan. This guideline recommended that *E.Coli* is not detected on 100mL of reclaimed water except for landscape irrigation uses (less than 1000CFU per 100mL for landscape irrigation).

Table 2.2 Water reclamation guidelines in Japan (MLITT, 2005)

Item	USES			
	Toilet Flushing	Sprinkling Water	Landscape Irrigation	Recreational Impoundment
E.Coli	N.D/100mL	N.D/100mL	< 1000CFU/100mL	N.D/100mL
Turbidity	< 2NTU	< 2NTU	< 2NTU	< 2NTU
pH	5.8 - 8.6	5.8 - 8.6	5.8 - 8.6	5.8 - 8.6
Color	-[a)]	-[a)]	< 40	< 10
Odor	Not discomfort	Not discomfort	Not discomfort	Not discomfort
Appearance	Not discomfort	Not discomfort	Not discomfort	Not discomfort
Residual chlorine	< 0.1mg/L	< 0.1mg/L	No restriction	< 0.1mg/L

a) Data will be set by consumers
N.D : Not Detected
NTU : Nephelometry Turbidity Unit

In Korea, water quality guidelines related with wastewater reuse were suggested through “wastewater reuse guidebook 2009” published by ME. This guideline suggests total 11 items of water qualities (pH, BOD, SS, turbidity, odor, color, residual chlorine, total fecal coliform, T-N, T-P, Cl^-) with respect to seven uses of reclaimed water (urban irrigation, landscape, river flow maintenance, recreational, groundwater recharge, agricultural, industrial, wetlands). In this guidelines, undetectable total fecal coliform per 100 mL is recommended except in case of uses for river flow maintenance, industrial and wetlands. This also recommend to meet that the concentration of total fecal coliform is less than 200CFU/100mL in case of uses for industrial and wetlands, and 1000 CFU/100mL for river flow maintenance.

2.2 contaminants related with public health risk in water reclamation

2.2.1 Waterborne pathogens

Waterborne disease outbreaks have been reported in worldwide (Craun et al., 2001; 2005; 2006; Dziuban et al., 2006; Yoder et al., 2008; Baldursson&Karanis, 2011). Waterborne disease are caused by pathogens such as bacteria, protozoa and viruses. The fecal pollution of water can lead to health problems because many of waterborne pathogens are intestinal microorganism (NRC, 1998). World Health Organization (WHO) estimated that 842,000 deaths per year is attributable unsafe water supply, sanitation and hygiene (WHO, 2014). In order to protect public health, therefore, the control of waterborne pathogens is important in water reclamation which uses wastewater as raw water. Table 2.3 shows major waterborne pathogens and diseases caused by them.

Table 2.3 Examples of major groups and genera of waterborne and water-based pathogens (Gerba, 1996; Straub&Chandler., 2003)

Group	Pathogen	Diseases caused
Bacteria	Salmonella	typhoid and diarrhea
	Shigella	diarrhea
	Campylobacter	diarrhea-leading cause in foodborne outbreaks
	Yersinia enterocolitica	diarrhea
	Escherichia coli O157:H7 and other certain strains	diarrhea, can lead to hemolytic uremia syndrome as a complication in small children.
	Legionella pneumophila	pneumonia and other respiratory infections
Protozoa	Naegleria	meningoencephalitis
	Giardia lamblia	chronic diarrhea
	Cryptosporidium parvum	acute diarrhea, fatal for immunocompromised individuals
	Cyclospora	diarrhea
	Entamoeba histolytica	amoebic dysentery
Helminths	Ascaris lumbricoides	ascariasis
	Trichuris trichiora	trichuriasis-whipworm
	Taenia saginata	beef tapeworm
Viruses	Enteroviruses (polio, echo, coxsackie)	meningitis, paralysis, rash, fever, myocarditis, respiratory disease, and diarrhea
	Hepatitis A and E	infectious hepatitis
	Norwalk viruses	diarrhea/gastroenteritis
	Astroviruses	diarrhea
	Sapporo	diarrhea/gastroenteritis
	Rotavirus	diarrhea/gastroenteritis
	Adenovirus	diarrhea (types 40 and 41), eye infections, and respiratory disease
	Reovirus	respiratory and enteric

2.2.1.1 Bacteria

Bacteria are single-celled prokaryotes that range in size from 0.2 to 10 micrometers (μm). Many type of bacteria are growth in the intestinal tract and then excreted in fecal matter. Among these bacteria, while the vast majority are harmless, several species are pathogenic and cause waterborne diseases. Even though classical waterborne bacterial diseases such as dysentery, typhoid, and cholera have decreased in the United States since the 1920s (Craun, 1991), it is still important because it has been reported that various waterborne disease such as campylobacteriosis, salmonellosis, legionellosis and etc are caused by pathogenic bacteria (Gerba et al., 1994; Grant et al., 1996; Wesley, 1996; Simon T., 1997). Campylobacter, nontyphoid Salmonella, and pathogenic Escherichia coli have been estimated to cause 3 million illnesses per year in the United States (Bennett et al., 1987).

2.2.1.2 Protozoa

Protozoa are single-celled eukaryotes which have generally larger in size than bacteria with animal-like behaviors, such as motility and predation. They range in size from 2 µm to 15 µm. It has reported that Giardia lamblia, Cryptosporidium parvum, and Entamoeba histolytica have been associated with gastrointestinal disease outbreaks in addition to Malaria one of the best-known disease caused by the genus Plasmodium (Feachem, D. G. et al., 1983; Craun, 1986; Bennett et al., 1987). In 1993, cryptosporidiosis outbreaks caused an estimated 400,000 illnesses and more than 50 deaths in Milwaukee and Wisconsin, U.S (Mac Kenzie et al., 1994; Hoxie et al., 1997).

Many protozoa produce spores, cysts or oocysts, which could be highly resistant to chlorine. Cryptosporidium oocysts and Giardia cysts of human origin are frequently detected in secondary wastewater effluent (Bitton, 2005). This presence of cysts or oocysts makes difficult to reduce the risk of infection from Cryptosporidium and Giardia through chlorine disinfection. Therefore, additional treatment processes are needed to prevent waterborne diseases by protozoa (NSC, 2012).

2.2.1.3 Helminths

Helminths are multicellular organisms that are visible to the naked eye. Helminths feed on a living host to receive nourishment and protection, while disrupting nutrient absorption of their hosts, causing weakness and disease. It has been reported that the prevalence of infection by helminths such as Ascaris lumbricoides occur mainly in developing countries (Ellis et al., 1993; Khuroo, 1996).

2.2.1.4 Virus

Viruses are intracellular infectious agents that replicates only inside the host. Viruses, which range in size from approximately 20 to 300 nanometers (nm), exist in the form of independent particles. These viral particles, known as virions, have either DNA or RNA as their genetic material and a protein coat, called the capsid, which surrounds and protects the DNA or RNA. Some virions have an envelope of lipids that surrounds the capsid. There are more than 120 identified human enteric viruses, including enteroviruses, rotaviruses, adenoviruses, astroviruses and Norwalk virus. Most enteric viruses cause gastroenteritis or respiratory infections, but some can lead to encephalitis, neonatal disease, myocarditis, aseptic meningitis, and jaundice (Gerba et al., 1985,

1996; Wagenkneckt et al., 1991).

2.2.1.4.1 Noroviruses

Noroviruses are a genetically diverse group of single-stranded positive-sense RNA, non-enveloped icosahedral viruses belonging to the *Caliciviridae* family (The Department of Health, 2006). Norovirus has one species, which is called Norwalk virus. Noroviruses were found in feces examined using electron microscopy in 1972 after an outbreak of acute gastroenteritis in Norwalk, Ohio in 1968 (Kapikian et al, 1972). Symptoms of Norovirus infection are vomiting, diarrhea and nausea.

Noroviruses genetically be classified into five genogroups (GI, GII, GIII, GIV and GV), which can be further divided into different genotypes. GI, GII and GIV infect humans, whereas GIII and GV infects bovine species and mice respectively (Smiley et al., 2003; Wobus et al., 2006; Zheng et al., 2006; Ramirez et al., 2008; Martella et al., 2008; Ntafis et al., 2010). It has been known that both a prevalence of norovirus and the detection in aquatic environmental tend to increase during winter seasons (Wstrell et al., 2006; da Silva et al., 2007; Haramoto et al., 2006; Harada et al., 2009; Belliot et al., 2010; Tran et al., 2010; Suzuki et al., 2011). Generally, it has been known that GII are the dominant genogroup and the cause of outbreaks or cases (Gallimore et al., 2007; Nguyen et al., 2008; Rodriguez-Diaz et al., 2009; Bruggink et al., 2010; Zheng et al., 2010). However, there are some reports that GI has a higher risk to cause infectious gastroenteritis because it is difficult to be removed during water treatment process, compare to GII (Clark et al., 2004; da Silva et al., 2007; Nordgren et al., 2009; Gentry et al., 2009). GIV has a few detection reports from both environmental and clinical samples (La Rosa et al., 2008; 2010; Kitajima et al., 2011; Sima et al., 2011).

2.2.1.4.2 Aichi virus

Aichi virus originally identified after an outbreak of acute gastroenteritis in Aichi, Japan in 1989 (Yamashita et al., 1991). Aichi virus is single-stranded positive-sense RNA, non-enveloped icosahedral viruses belong to Picornaviridae family (Yamashita et al., 1998; Reuter et al., 2011; Knowles et al., 2012). Aichi virus cause vomiting, diarrhea and stomach cramps (Drexler et al., 2011).

Aichi virus is divided into three genotypes, A, B and C (Yamashita et al., 2000; Ambert-Balay., 2008). The detection of Aichi virus in the environmental or wastewater samples has been reported (Alcala et al., 2010; Sdiri-Loulizi et al., 2010; Kitajima et al., 2011;

2013;). A is dominant genotype in Japan and Tunisia, according to previous researches, whereas B predominate in Venezuela, Germany and Southeast Asia (Kitajima et al., 2011; Drexler et al., 2011).

2.2.1.4.3 Pepper Mild Mottle Virus

Pepper Mild Mottle Virus (PMMoV) is a rod-shaped virus, approximately 18nm in diameter and 300nm in length (Wetter et al., 1984; King et al., 2011). PMMoV is non-enveloped, single-stranded positive-sense RNA virus belong to classified in the genus Tobamovirus, Virgaviridae family (Wetter et al., 1984; Colson et al, 2010). PMMoV infects various solanaceous plants, such as capsicum, tomato, and tabacco (King et al., 2011). There have been some reports that PMMoV is present in human feces and has a potential to be used as an indicator of human fecal pollution in water (Rosario et al., 2009; Colson et al., 2010; Hamza et al., 2011; Haramoto et al., 2013; Han et al., 2014). The ingestion of foods containing pepper or capsicum is known to be major sources in human feces (Zhang et al., 2006). PMMoV was detected as concentrations in 10^8~10^{10} and 10^5~10^{10} copies/L from raw and treated wastewater, respectively (Rosario et al., 2009; Hamza et al., 2011; Kitajima et al., 2014). PMMoV was not only more abundant, but also more persistent in water samples than human adenoviruses and human polyomaviruses (Hamza et al., 2011). These properties indicate the potential of PMMoV as an indicator of virus in spite of a morphological difference between PMMoV and human enteric viruses.

2.2.1.4.4 Bacteriophages

Bacteriophages are a virus that infects and replicates in bacteria. 19 families of bacteriophages are currently recognized by the International Committee on Taxonomy of Viruses (ICTV) (King et al., 2011). Bacteriophages belong to Podoviridae, Myoviridae and Styloviridae have double-stranded DNA, and others belong to Inoviridae, Microviridae have single-stranded DNA, and others belong to Leviviridae have single-stranded RNA (Matthews et al., 1982; IAWPRC, 1991). In addition, some bacteriophages called somatic phage infect the bacteria via the cell wall and others called F-specific phage infect via the F-pilus. Bacteriophages not only exhibit morphological similarities with human enteric virus but also can be easily and rapidly cultivated in laboratories. Various bacteriophages such as T4 and φX174 (somatic phage), and MS2 and Qβ (F-specific phage), for these reasons, have been used as models or surrogates for the fate

of human enteric viruses in the environment and water treatment through many researches (Cole et al., 2003; Farahbakhsh et al., 2004; Matsushita et al., 2005; Fiksdal et al., 2006; Nappier et al., 2008; Shirasaki et al., 2009a; 2009b; Boudaud et al., 2012; ElHadidiy et al., 2013; Haramoto et al., 2015; Vergara et al., 2015).

Among many bacteriophages, F-specific phage which belong to Leviviridae family are non-enveloped icosahedral viruses with single-stranded RNA, and are 24 – 36nm in diameter (King et al., 2011). They are called FRNA phages (FPH), and widely used as models or surrogates for human enteric viruses. FPH divided into four genogroups (GI, GII, GIII and GIV). GI and GII are belong to the genus Levivirus, whereas GIII and GIV are belong to the genus Allolevivirus. MS2 is a representative strain of GI, and GA, Qβ and SP are the representative strains of GII, GIII and GIV respectively. Furthermore, GI and GIV are generally detected in animal feces while GII and GIII are generally detected in human feces (Osawa et al., 1981; Furuse et al., 1981; Hsu et al., 1995; Cole et al., 2003). However, there are few reports that GI and GII is also detected from human feces and animals feces respectively (Hsu et al., 1995; Cole et al., 2003).

Some research suggested that there are a difference in persistence of FPH in environment or during water treatment depending on their genogroup (Cole et al., 2003; Niapper et al., 2006; Boudaud et al., 2012; Haramoto et al., 2012; Hata et al., 2013 Yang et al., 2013). GI-FPH is most resistant to wastewater treatment, compared with other genogroups (Hata et al., 2013; Haramoto et al., 2015). However, it was also reported that GA, a representative strain of GII-FPH, is most resistant to drinking water treatment using ultrafiltration membrane (Boudaud et al., 2012). Qβ, a representative strain of GIII-FPH, is easily inactivated during coagulation than MS2 (Matusi et al., 2003; Shirasaki et al., 2009; Matsushita et al., 2011). GIV-FPH is scarcely detected in aquatic environment and human feces (Ogorzaly and Gantzer 2006; Love et al. 2008; Wolf et al. 2008; Ogorzaly et al. 2009; Hata et al., 2013).

2.2.2 Disinfection by-products

Chlorination disinfection has been introduced into water treatment in order to inactivate pathogenic microorganisms since the early 1990s. An outbreak of infectious waterborne diseases such as typhoid and cholera is dramatically decease since starting chlorination disinfection in drinking water. However, it was discovered that by-products such as trihalomethanes (THMs) can be formed during chlorination disinfection (Rook, 1974). In 1976, the US National Cancer Institute (USNCI) published that chloroform is linked to cancer from the result using laboratory animals (USNCI, 1976). As a result, a public

health issue related with disinfection by-products (DBPs) was recognized. Various DBPs over 600 have been identified including N-nitrosamines, haloacetic acids (HAAs) and aldehydes (ADHs) (Richardson et al., 1998; 2008; 2011).

There are few reports with regard to the influence of effluent organic matter (EfOM) on DBPs formation (Sirivedhin and Gray, 2005). For this reason, the formation of DBPs during wastewater treatment has also been studied because it is possible to threaten aquatic organisms or the public health of drinking water users who live in downstream (Wert et al., 2007; Hollender et al., 2009; Tripathi et al., 2011; Zimmermann et al., 2011).

2.2.2.1 THMs

THMs were the first DBPs identified and one of most prevalent classes of DBPs formed in chlorinated water (Bellar et al., 1974; Rook et al., 1974; Krasner et al., 2006). THMs are chemic compounds in which three of the four hydrogen atoms of methane are replaced by halogen atoms. THMs are not regulated individually by the U.S. EPA. There is only regulation for total trihalomethanes at a level of 80 μg/L (U.S. EPA, 2006). WHO set guideline values for individual THMs in drinking water (80 μg/L for bromodichloromethane, 100 μg/L for bromoform, 300 μg/L for chloroform, 100 μg/L for dibromochloromethane, respectively). According to The International Agency for Research on Cancer (IARC), both chloroform and bromodichloromethane are classified as possible human carcinogens (Group 2B) whereas bromoform and dibromochloromethane are not classifiable as to their human carcinogenicity (Group 3) (IARC, 1991; 1999a; 1999b).

2.2.2.2 ADHs

ADHs (and ketones) are organic compounds which incorporate a carbonyl functional group. Although chlorine and chlorine dioxide treatment can also form low ppb level of formaldehydes (Dabrowska et al., 2003; Richardson et al., 2003; 2007), aldehydes are produced primarily by ozonation. (Glaze et al., 1987; Schechter et al., 1995; Gagnon et al., 1997; Kuo et al., 1998; Richardson et al., 1998; Can&Gurol, 2003). Bromate is the only ozone DBPs regulated in drinking water by U.S. EPA (a maximum contaminant level (MCL) of 10 μg/L). In Japan, formaldehydes is also regulated at 80 μg/L in addition to bromates (Japanese Ministry of Health, Labour and Welfare, 2010). Formaldehyde and acetaldehyde are classified as human carcinogens (Group 1) and possible human carcinogens (Group 2B) by IARC, respectively (IARC, 2006). There are several reports

that formaldehyde and acetaldehyde have been shown to be carcinogenic in rodents when administered through inhalation, but were not carcinogenic when administered through drinking water (Swenberg et al., 1980; Kerns et al., 1983; Soffritti et al., 2002).

2.2.2.3 N-nitrosamines

N-nitrosamines are chemical compounds that a nitroso group bonded to an amine. *N*-nitrosamines include *N*-nitrosodimethylamine (NDMA), *N*-nitrosomethylethylamine (NMEA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodipropylamine (NDPA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosopiperidine (NPIP), *N*-nitrosomorpholine (NMOR) and *N*-nitrosodi-n-butylamine (NDBA). Although NDMA are produced primarily by chloramine disinfection, recent studies indicate that the formation also occurs during ozonation (Schmidt and Brauch, 2008; Andrzejewski et al., 2008; Kosaka et al., 2009; Hollender et al., 2009; von Gunten et al., 2010; Pisarenko et al., 2012; Marti et al., 2015; Gerrity et al., 2015). NDMA has been classified as probable human carcinogens by both U.S. EPA and IARC (IARC, 1987; U.S. EPA, 1993). CDPH established a notification level of 10ng/L for NDMA, NDEA and NDPA (CDPH, 2011). According to WHO guideline for drinking water quality, a guideline value of NDMA is 100ng/L (WHO, 2011). In addition, guideline values of 10 ng/L for NDMA, 10 ng/L for NDEA and 1 ng/L for NMOR have been established in the Australian Guidelines for Water Recycling (Natural Resource Management Ministerial Council, 2008). Canada has established a 40 ng/L maximum acceptable concentration for NDMA (Health Canada, 2011)

2.3 water treatment technologies for water reclamation

2.3.1 ozonation

Ozone is a powerful an oxidizing agent that has been widely used for drinking water or wastewater treatment. It has been well documented that ozonation is effective to remove Endocrine Disruptive Chemicals (EDCs) and Pharmaceutical and Personal Care Products (PPCPs) in addition to colors and odors (Zwiener and Frimmel, 2000; Huber et al., 2005; Snyder et al., 2006; Nakada et al., 2007; Kim and Tanaka, 2010; Yang et al., 2011). Moreover, ozonation can inactivate most waterborne pathogens including giardia and Cryptosporidium difficult to inactivate by chlorine disinfection (Facile et al., 2000; Xu et al., 2002; Fang et al., 2014). There are few reports related with virus inactivation using

ozonation (Kim et al., 1980; Roy et al., 1981; Tyrrell et al., 1995; Shin et al., 2003; Fang et al., 2014). Ozone first destroys viral capsids and then liberated RNA are damaged (Kim et al., 1980). Recently, it has been revealed that ozonation is effective to alleviate membrane fouling when it used as pretreatment of membrane filtration (Lee et al., 2005; Kim et al., 2008; Geluwe et al., 2011; Zhang et al., 2013; Fan et al., 2014; Cheng et al., 2016). According to these researches, the mitigation of membrane fouling is result from the degradation of organic matters by ozonation (Lee et al., 2005; Wang et al., 2007; Kim et al., 2008). Ozonation was able to mitigate reversible fouling in consequence of the degradation of high molecular weight (MW) hydrophobic biopolymers to low MW and more hydrophilic compounds. However, ozonation had a limited effect on mitigation of irreversible fouling dominated by the high MW hydrophilic organic matters (Zhang et al., 2013; Wei et al., 2016).

2.3.2 Coagulation and membrane filtration

A membrane is a physical barrier that permit the passage of materials only up to a certain size, shape. Membranes are classified as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) membranes in accordance with their pore size (Figure 2.5). Membranes is also able to be classified on the basis of their materials or module structures. A majority of membranes industry accounts for membranes manufactured from natural or synthetic polymers, known as organic membrane. However, an inorganic membrane made from inorganic materials such as alumina, titania and silica has been received attention recently. Inorganic membranes usually have higher thermal and chemical stability in addition to durability compared with organic membrane. These properties make them usable under high temperature or pressure conditions, and also be able to endure backwashing using powerful acids and bases.

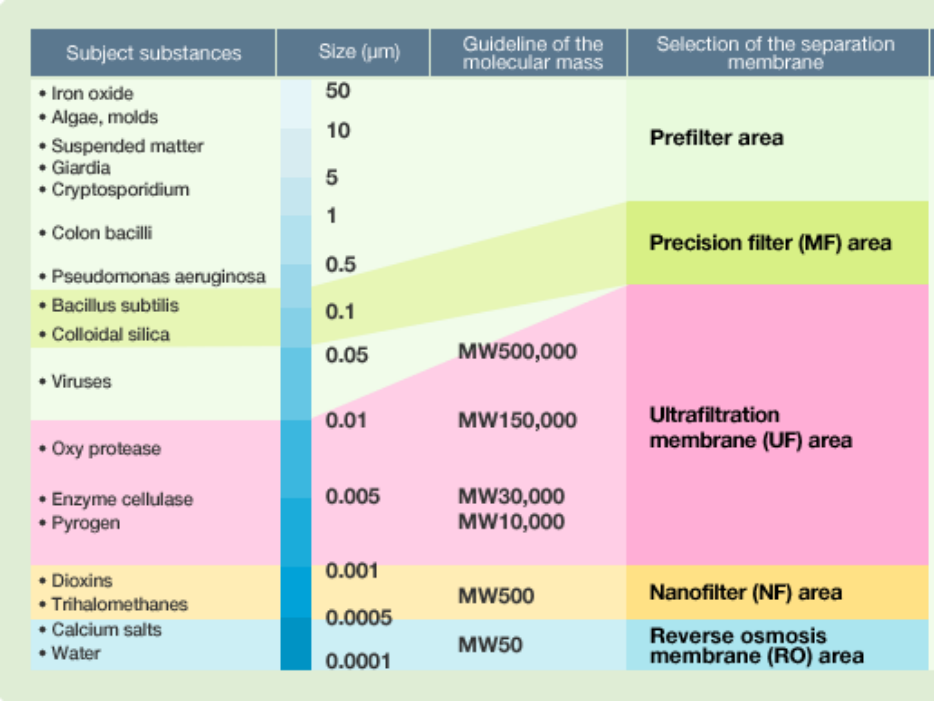


Figure 2.5 Membranes types and their subject substances

(Source : Daicen membrane systems homepage)

Membrane filtration has been considered as one of most effective water treatment technologies for water reclamation because it is able to perfectly remove various contaminants such as suspended solids (SS), bacteria and organic particles which have bigger size than pore size. However, membrane fouling, which causes severe performance deterioration of membrane, still remains as the biggest technical challenge with regard to the use of membranes for water reclamation. Membrane fouling can be divided into hydraulically reversible fouling and irreversible fouling, depending on whether it is easy to be removed by hydraulically backwashing or not (Kimura et al., 2006; Yamamura et al., 2007; Zhu et al., 2012). Hydraulically irreversible fouling is much difficult to be removed than reversible fouling because it is only eliminated by chemically enhanced backwashing (CEB), hence the mitigation of irreversible fouling is one of the challenges on membrane filtration.

Many research suggested that the irreversible fouling of membranes used for wastewater treatment is mainly due to colloids, natural organic matter (NOM) or EfOM (Jarusutthirak et al., 2001; Shon et al., 2006a, 2006b; Yamamura et al., 2007; Zhu et al., 2010). These foulants such as NOM or EfOM are usually small particles and negatively charged, so they do not aggregate and settle due to a repulsive force between themselves. In order to alleviate irreversible fouling, it is needed to form aggregates

which are easily removed by sedimentation or membrane filtration through processes adding coagulant positively charged. For these reasons, coagulation has been widely used as pretreatment of membrane filtration in order to mitigate membrane fouling and improve filtered water quality (Peuchot et al., 1992; Lee et al., 2000; Carroll et al., 2000; Judd et al., 2001).

In addition, many researches related with virus removal using coagulation and membrane filtration have been conducted. (Nasser et al., 1995; Fiksdal et al., 2006; Shirasaki et al., 2009; Matsushita et al., 2013). According to previous researches, it was difficult to remove viruses by membrane filtration only because viruses are much smaller (1~300nm) than membrane pore size in some cases. MF or UF membrane filtration achieved 0 ~ 2.5-log removal rate in MS2 spike experiments (Farahbakhsh et al., 2004; Fiksdal et al., 2006; Huang et al., 2012). However, it is possible to improve virus removal performance of membrane filtration when coagulation are used as pretreatment (Zhu et al., 2005a, 2005b; Matsushita et al., 2006; Fiksdal et al., 2006; Guo and Hu., 2011). According to recent researches, furthermore, virus was inactivated during coagulation and flocculation (Matsui et al., 2003; Shirasaki et al., 2009; Guo and Hu, 2011; Matsushita et al., 2011, Kreißel et al., 2014). They reported that the specific spices in polyaluminum chloride (PACl) probably played a major role in virus inactivation during coagulation (Kreißel et al., 2014; Shirasaki et al., 2016).

2.3.4 Ozonation and ceramic membrane filtration combination process

Membrane filtration, as mentioned before, has a disadvantage to the removal of viruses or PPCPs whereas it can remove perfectly the SS or bacteria. On the other hand, ozonation is effective to remove PPCPs or dissolved organic matters. Moreover, ozonation is able to not only alleviate membrane fouling but also inactivate viruses. According to previous researches, membrane fouling was mitigated with incorporating ozonation as pretreatment for membrane filtration (Kim et al., 2008; Park et al., 2010; Van Geluwe et al.,2011; Zhang et al., 2013; Fan et al., 2014; Cheng et al., 2016; Wei et al., 2016). Thus, it was expected that much higher quality reclaimed water is possible to be produced more efficiently through the development of ozonation and ceramic membrane filtration combination process (O_3&CMF process). However, organic membrane could be damaged by residual ozone in case that ozonation was used as pretreatment for membrane filtration. Therefore, ceramic membrane is more suitable to the combination with ozonation because it is able to operate under high pressure condition, and also endure residual ozone which has a powerful oxidative ability.

2.4 Position of this research

As mentioned before, membrane fouling could be mitigated by incorporating ozonation as pretreatment for membrane filtration. However, there is insufficient information regarding long term operational performance of O_3&CMF process. Although Lehman et al. (2009) reported stable performance for approximately 680 hours at a flux of 4 m/d with pretreatment using ozoantion (4 mg/L) and coagulation (1 mg-Al/L), this operation was conducted under the one operational condition and without chemical enhanced backwashing (CEB). In addition, they have not provided any information with regard to virus removal performance and directly linked with health risk of reclaimed water users. Therefore, it is necessary to evaluate not only operational performance through long term operation, but also virus removal performance of O_3&CMF process. In terms of virus removal performance, it was evaluated through spike test using MS2 as a model virus, and also occurrence and the removal of indigenous virus was investigated based on reverse transcription - quantitative polymerase chain reaction (RT-qPCR) assays. To complement RT-qPCR which provided information regarding only the presence or absence of specific DNA/RNA sequence regardless of whether viruses retain infectivity, the infectivity of indigenous viruses was evaluated through the application of RT-PCR based genotyping after FPH propagation in liquid medium (integrated culture [IC]-RT-PCR) (Hata et al., 2016). Moreover, ozonation can produce various DBPs, and it is required to rigidly control DBPs depending on the uses of reclaimed water. In this study, the formation of DBPs during O_3&CMF process was investigated, and the addition of biological activated carbon (BAC) treatment was considered to control DBPs. Although ozonation followed by BAC has been widely used as an advanced drinking water treatment technology, the effect of BAC on membrane fouling is still unclear. Therefore, the effect on not only the removal of DBPs but also ceramic membrane filtration caused by adding BAC to O_3&CMF process were also investigated.

In addition, it is difficult to ensure the reliability of water treatment performance because treatment performance could be variable depending on the fluctuation of source water quality. For this reason, a novel water treatment monitoring systems, which make it possible to take action instantly when treatment fails, is needed. Although several monitoring indicators for the formation of DBPs has been reported (Hua et al., 2007; Gerrity et al., 2012; Liu et al., 2012; 2015), but there are no reports in terms of monitoring indicator for both virus removal and the formation of DBPs at the same time. In this study, thus, a novel water treatment monitoring systems, which can predict both virus removal and the formation of DBPs would be suggested to develop of O_3&CMF process which

consistently provide hygienic safety reclaimed water.

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Chapter Ⅲ

Evaluation of each process and the selection of efficient process sequence for treating secondary and primary effluent

3.1 Introduction

As mentioned in Chapter II, ceramic membrane filtration (CMF) has been received attention as a treatment technology for water reclamation in terms of their superior durability and the perfect elimination of various contaminants such as suspended solids (SS), bacteria and organic particles which have bigger size than pore size. However, viruses which have smaller size than pore size is difficult to be removed by only membrane filtration (Jacangelo et al., 1995; Madaeni et al., 1995; Fiksdal et al., 2006). Also, membrane fouling, which causes severe performance deterioration of membrane, still remains as the biggest technical challenge. In order to complement these technical challenges, ozonation and coagulation have been considered as pre-treatment for CMF. It has been reported that coagulation is helpful to not only enhance the virus removal performance of membrane filtration (Zhu et al., 2005a, 2005b; Matsushita et al., 2006; Guo and Hu, 2011), but also mitigate membrane fouling (Lee et al., 2000; Carroll et al., 2000; Judd et al., 2001). Meanwhile, it has well known that ozonation could inactivate various pathogens including viruses (Tyrrell et al., 1995; Shin et al., 2003; Fang et al., 2014; Sigmon et al., 2015). Also, there are several reports that membrane fouling was mitigated with incorporating ozonation as pretreatment for membrane filtration (Kim et al., 2008; Park et al., 2010; Van Geluwe et al.,2011; Zhang et al., 2013; Fan et al., 2014; Cheng et al., 2016; Wei et al., 2016). However, there are only a few studies that both ozonation and coagulation were applied to membrane filtration, contrary to many

previous researches that investigated the application of each ozonation and coagulation as pretreatment separately.

Ozonation and ceramic membrane filtration combination process (O_3&CMF process) could largely divide into two types of process sequence; ozonation followed by CMF (O_3+PACl+CMF) and CMF followed by ozonation (PACl+CMF+O_3). Pre-ozonation could mitigate membrane fouling, but the change of water quality by pre-ozonation might influence subsequent coagulation. According to previous researches, pre-ozonation acted as a coagulation aid for the removal of natural organic matter (NOM) until a certain level of ozone dosage, while it was detrimental to NOM removal and coagulated floc size at higher ozone dosage (Yan et al., 2007; Li et al., 2009). On the other hand, post-ozonation could improve the efficiency of ozonation, which is lead to the reduction of ozone dosage.

However, this treatment efficiency depends on the source water quality and the target water quality for reclaimed water use. In this study, both secondary effluent (SE) and primary effluent (PE) are considered as source water, and there is a huge difference between the water quality of SE and PE. Accordingly, efficient process sequence might also be different.

In this chapter, therefore, the virus removal performance of both ozonation and coagulation was evaluated through lab scale experiment, and also the effect of pretreatment on subsequent treatment was investigated, considering process sequence in O_3+PACl+CMF and PACl+CMF+O_3. Moreover, the amount of energy required to achieve target virus removal rate according to reclaimed water uses by O_3&CMF process was calculated. On the basis of these performance evaluation and the assessment of energy consumption, ultimately, the efficient process sequence in accordance with source water was decided prior to the continuous operation of O_3&CMF process in chapter IV.

3.2 Materials and Methods

3.2.1 Procedure for the selection of efficient process sequence

Figure 3.1 illustrates the schematic diagram of this chapter.

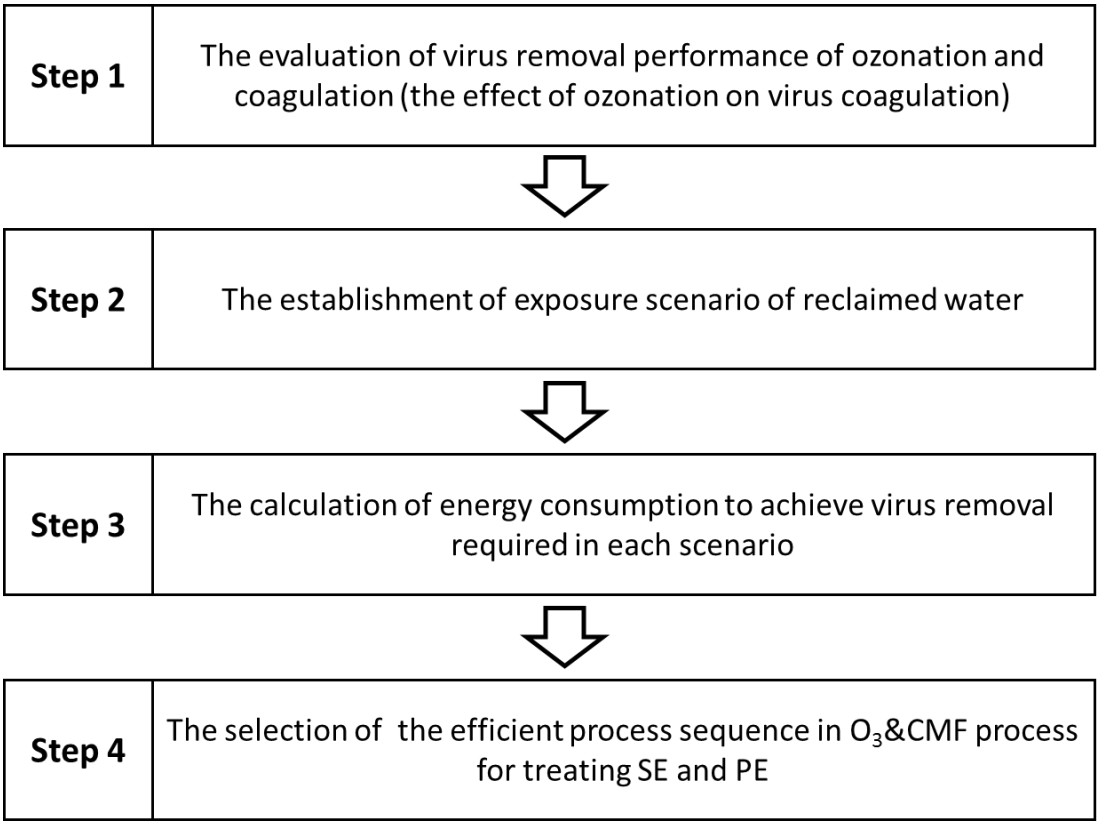

Figure 3.1 Schematic diagram of this chapter

3.2.1 Water quality analysis

Total organic carbon (TOC), dissolved organic carbon (DOC), pH and zeta-potential were analyzed as water quality items. Samples for DOC analysis were filtered through GF/B filter (pore size 1.0μm, Cat No. 1821-047, Whatman) prior to measurement. TOC and DOC were measured by TOC analyzer (TOC-5000A, SHIMAZU; TOC-300V, Mitsubishi Chemical Analytech). Zeta potential, an indicator of the stability of colloidal dispersion, was measured by Zeta potential analyzer (Zetasizer Nano ZS, Malvern).

3.2.2 Bacteriophage

3.2.2.1 Preparation of bacteriophage suspensions

In this study, F-specific bacteriophage MS2 (NBRC102619) was obtained from NITE (National Institute of Technology and Evaluation) Biological Research center (NBRC, Japan). MS2 has a single-stranded RNA which is encapsulated in an icosahedral capsid with a diameter of 24-26 nm. These morphological properties of MS2 are similar with

human enteric viruses. For this reason, MS2 is widely used as a surrogate in order to evaluate virus removal performance of water treatment process.

MS2 was propagated according to standard procedure (ISO 10705-1) using *Escherichia coli* K12 (NBRC 13965) as bacterial host. After propagation, the MS2 suspension was centrifuged (3000 rpm for 15 min) and the supernatant filtered through a membrane filter (pore size 0.45 μm, cellulose acetate; ADVANTEC). The filtered MS2 suspension was stored as stock suspension at 4℃ until experiments. The final concentration of MS2 stock suspensions was 10^9 ~ 10^{11} PFU/ml.

3.2.2.2 Sample pretreatment

Sample pre-treatment was conducted in accordance with method of establishing reference (Lee, 2015). Figure 3.2 describes the sample pre-treatment flow.

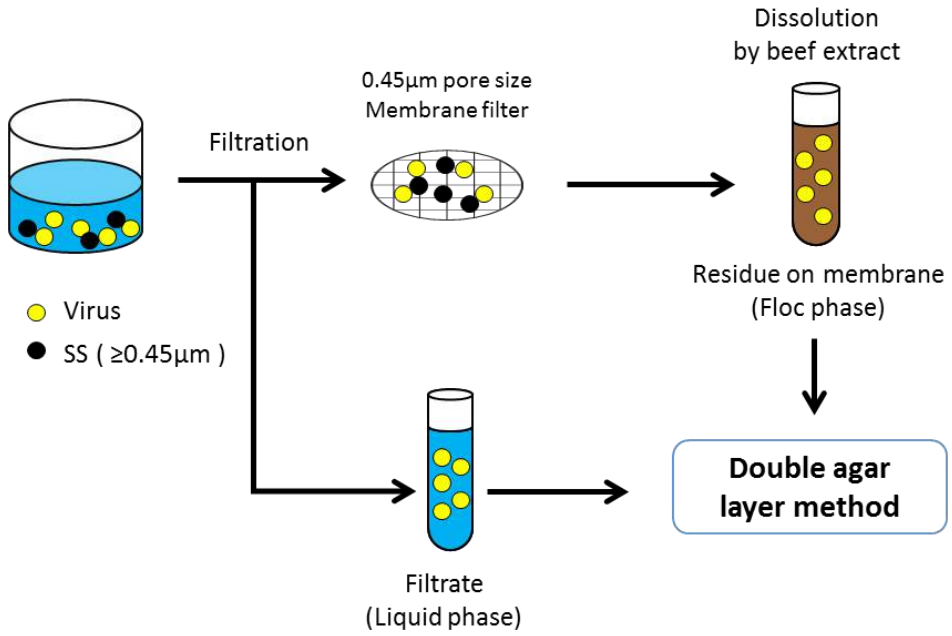

Figure 3.2 Sample pre-treatment flow for MS2 analysis (Lee, 2015)

During coagulation, although most of MS2 were entrapped in the aluminum floc particles, some of MS2 remained suspended in the liquid phase. Samples were divided by membrane filter in order to analyze MS2 in both the floc phase and the liquid phase. Frist of all, samples were passed through 0.45 μm membrane filter to analyze MS2 entrapped in the floc particles during coagulation. The results of the filtrate and the residue on membrane filter were regarded as MS concentration in the liquid and the floc

phase, respectively. The residue on membrane filter were dissolved by 3% beef extract adjusted to pH 9.5 with NaOH and vortexed for 3min. Beef extract was used in an effort to prevent the inactivation of MS2 during floc dissolution (Matsui et al., 2003). After dissolution, the beef extract was filtered using syringe filter (0.45μm pore size, ADVANTEC) once again. Each beef extract and filtrate were analyzed using double agar layer method after the appropriate dilution with liquid medium.

3.2.2.3 Bacteriophage analytical methods

MS2 was analyzed according to double agar layer method (ISO 10705-1) using the bacterial host *Escherichia coli* K12. Table 3.1 shows composition of medium for double agar layer method. Sample of 1 ml was added upon a prepared bottom agar layer on petri dishes, and then top agar layer containing the bacterial host was poured. The petri dishes were then incubated for 18 ~ 24 hours at 37℃. The number of plaque counts in petri dishes was considered as the infectious MS2 concentration, which was expressed in plaque forming units per milliliter (PFU/ml). The petri dishes were prepared in duplicate and the concentration was averaged from the plaques counted on each petri dish. Sample of 50 ml was examined if it was expected that samples had low MS2 concentration. Sample of 10 ml was poured onto each of 5 petri dishes with 10mL of 2x medium containing the bacterial host. The detection limit was about 0.5 PFU/mL (1ml of tested volume) and 0.02 PFU/mL (50ml of tested volume) in this experiment.

Table 3.1 Composition of medium

	Top medium	Bottom medium	2 x medium	Liquid medium
	g/1000ml-Milli-Q			
LB Broth	20	20	40	20
Bacto Agar	8	11	16	-
$CaCl_2 \cdot 2H_2O$	1	1	2	-

3.2.3 Calculation of virus removal rate

Virus removal in these experiments is expressed as a log removal value according to the following Eq. 3.1 (EPA, 2001).

$$\text{MS2 log removal rate} = \log\left(\frac{C_o}{C}\right) \quad \text{(Eq. 3.1)}$$

Where, C_0 is MS2 concentrations in source water, C is MS2 concentrations in ozonated water or supernatant after coagulation.

MS2 removal rate was calculated using MS2 concentration in both floc phase and liquid phase. In the coagulation experiment, however, virus removal rate was calculated using only MS2 concentration in liquid phase. MS2 in floc phase would be removed perfectly by ceramic membrane (pore size 0.1μm) when coagulation was followed by ceramic membrane. It is important that MS2 in liquid phase transferred to floc phase as much as possible in order to increase MS2 removal by coagulation and ceramic membrane filtration. Therefore, MS2 removal rate was evaluated using MS2 concentration in liquid phase transferred to floc phase during coagulation.

3.2.4 Experimental setup and methods

3.2.4.1 Ozonation

In ozonation experiment, secondary effluent, primary effluent and their CM permeates was used as a source water. MS2 was spiked into source water, and its initial concentration was approximately 10^6 ~ 10^7PFU/ml. Ozonation experiments were conducted using a semi-batch cylindrical reactor (Figure 3.3). The temperature of source water was maintained at 20℃ by circulating water from a water temperature controlling system into a water jacket outside the reactor. Ozone gas was generated by an ozone generator (POX-10, Fuji electronics). The generated ozone gas was fed into the reactor filled with source water continuously. The concentration of generated ozone, and excess ozone was measured by ozone monitors (EG-600, Ebara, OZ-20, DKK-Toa). During ozonation, the water was mixed by a mechanical agitator. The residual ozone in collected samples was quenched by sodium thiosulfate.

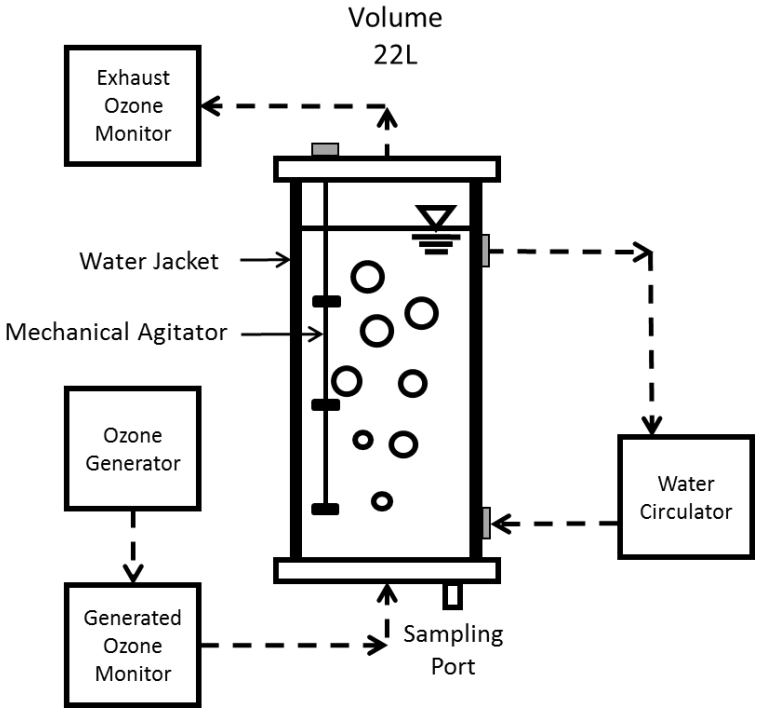

Figure 3.3 Semi-batch reactor for ozoanation

3.2.4.2 Coagulation

MS2 removal by coagulation and sedimentation (CS) was investigated through Jar-test. Secondary effluent, primary effluent and their ozonated water were used as source water. Ozonated water was obtained according to the method described in 3.2.4.1. Jar-test was conducted after quenching residual ozone. The pH of ozonated water was adjusted to be match with SE or PE (around 7) by HCl or NaOH.

Source water of 1 L in 1L beakers was spiked with MS2 stock solution to be final concentration of 10^6~107PFU/ml, and then polyaluminium chloride (PACl, 10~11% of Al2O3, Takasugi pharmaceutical) was added as a coagulant. Source water in beakers was stirred for 5 min at 150rpm (G=615s-1, rapid mixing) and then for 5 min at 50rpm (G=118.5s-1, slow mixing) using a jar-tester (MJS-4H, Miyamoto Manufacturing). The water was then left at rest for 30 min to settle the floc particles. Samples were taken from supernatant after sedimentation except for samples for zeta potential analysis immediately collected after mixing.

3.2.4.3 Experimental setup of coagulation and ceramic membrane filtration

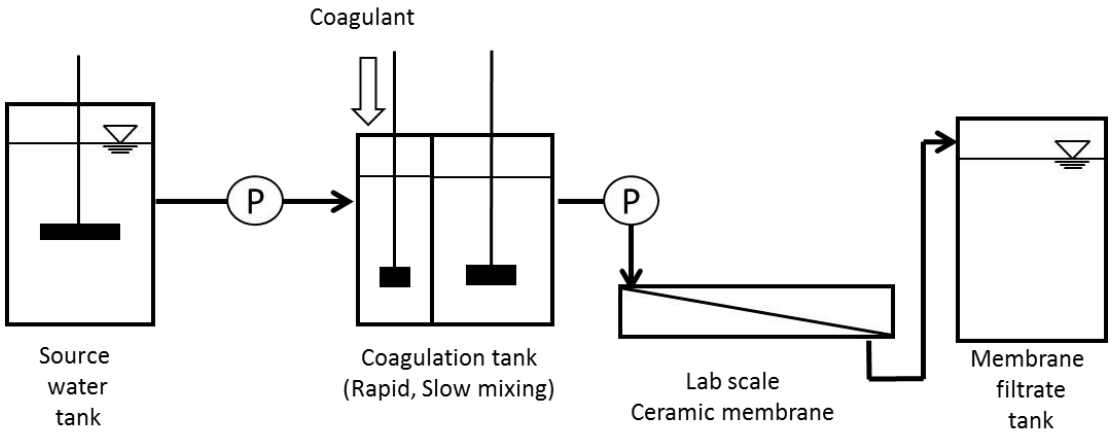

Figure 3.4 Experimental setup of lab scale ceramic membrane

Table 3.2 Characteristics of lab scale membrane

Monolith ceramic membrane (Internal pressure)	
Membrane module size	ϕ 30mm × 100mm
Channel Number	55 channels
Effective Area	0.042m^2
Pore Size	0.1μm

Figure 3.4 describes the experimental setup of lab scale ceramic membrane. Secondary and primary effluent was used as source water in this experiment. The turbidity of source water was monitored by a turbidity meter (DTS-12, Environmental system). The source water flowed to a coagulation tank at constant flow rate (400mL/min). The coagulation tank consisted of rapid (G=659 s^{-1}, a retention time : 2.5 min) and slow mixing (G=393 s^{-1}, a retention time : 5.5 min) part. PACl (10~11% Al_2O_3, Takasugi pharmaceutical) was used as coagulant, and injected in the rapid mixing tank. The coagulated water fed into the ceramic membrane filtration. The filtration for treating SE was operated at the constant flux 4 m/d (116 mL/min) in a dead-end mode and continued for 60 min. In case of treating PE, the operation was conducted at the constant Flux 2 m/d (58 mL/min) in a dead-end mode and continued for 20 min. At the end of each filtration cycle, ceramic membrane was backwashed at a pressure of 450kPa with the filtrate for 10s, and was followed by an air blow with compressed air at a pressure of 300kPa. The collected filtrate was subjected to ozonation experiment using the semi-batch ozone reactor.

3.2.5 Energy consumption calculation

3.2.5.1 Ozonation

In this study, energy consumption for ozonation was calculated by referring to the assumption in previous research (Munoz et al., 2009), and the detail was described as follows. O_3 generator with a capacity of 1 kg O_3/h was used, and ozone reactors had a gas-to-liquid transfer efficiency of 75%. This assumption included power consumption for producing O_3, pumping and excess ozone destruction. Energy consumption required to produce 1 kg of O_3 was 15.85 kWh. This ozonation system could treat at maximum of 4000m^3/d under 6 mg/L of ozone dosage. Thus, the capacity of water reclamation treatment plant was assumed as 4000m^3/d in this chapter.

3.2.5.2 Coagulation

Energy consumption for mixing in coagulation tank was calculated in accordance with the formula proposed by Camp and Stein (1943).

$$E_m = G^2 \times \mu \times T \qquad \text{(Eq.3.2)}$$

Where, E_m is energy consumption for mixing (Wh/m^3), G is velocity gradient (S^{-1}), μ is dynamic viscosity of wastewater (1.005 x 10^{-3} Pa×s at 20℃) and T is coagulation time (h). In this study, a two-step mixing was conducted (rapid mixing and slow mixing) with G values of 615 s^{-1} and 118.5 s^{-1}, respectively. Each mixing step had 5 min of coagulation time. Consequently, E_m was 3.3 x 10^{-2} kWh/m^3.

The energy consumption for coagulants was calculated as follows: a carbon footprint for PACl production was 0.537 kgCO_2/kgPACl (ICOPA, 2014). This carbon footprint factor was converted to energy consumption based on a carbon footprint of electricity generation. The carbon footprint factor of electricity generation was 0.555 kgCO_2/kWh (Editing committee of LCA practical guide, 1998), and therefore the energy consumption of PACl was 0.968 kWh/kgPACl.

3.2.5.3 CMF

Energy consumption for CMF was determined by referring to the assumption in previous research (Wang, 2013). This assumption included electricity consumption and chemical cost, required for CMF operation and chemical enhanced backwash (CEB), respectively. In this chapter, however, only electricity consumption was considered as running energy of CMF, and the chemical cost was excluded. Energy consumption of CMF including chemicals cost for CEB would be reconsidered in chapter IV, based on the result of long term operation.

Energy consumption of CMF was 0.0292 kWh/m^3 in this assumption. It was calculated by electricity consumption of both main pump and air compressor, because the electricity was primarily consumed for them during CMF operation.

3.2.6 Quantitative Microbial Risk Assessment

Quantitative Microbial Risk Assessment (QMRA) was used to estimate potential adverse health effects associated with exposure of virus to human. In this study, norovirus was selected as a model virus. It has been well reported that noroviruses cause viral gastroenteritis (Gallimore et al., 2007; Nguyen et al., 2008; Rodriguez-Diaz et al., 2009; Bruggink et al., 2010; Zheng et al., 2010). The detail of calculation was as follows; Firstly, five exposure scenarios including recreational impoundment, municipal irrigation, garden irrigation, toilet flushing and crop irrigation were decided according to previous reports (Tanaka et al 1998; NRMMC et al.,2006) (Table 3.2). Secondary, disability adjusted life years (DALYs) was calculated using Eq.3.3 to assess disease burdens when users exposure to reclaimed water. The probability of infection ($P_{inf}(D)$) was calculated using dose-response relationship of norovirus (Teunis et al., 2008) (Eq.3.4). The probability of illness conditional on infection (R_{inf}) was reported as 0.8 (Teunis et al., 1996). Disease burden (DB), the impact of a health problem as measured by financial coast, mortality, morbidity or other indicators, was decided as 9.0 x 10-4, referring to a previous report (Duizer et al., 2004; Kemmeren et al., 2006). Thirdly, the acceptable concentration of norovirus in reclaimed water was estimated. The acceptable level of risk was defined as less than 10^{-6} DALY per person per year, as high as level of the acceptable risk in drinking water set by WHO (WHO, 2004). The acceptable concentration of norovirus was calculated by Eq. 3.3. Finally, log removal of norovirus required for each scenario was calculated using in accordance with Eq. 3.1. The concentration of norovirus in source water was derived from the result in Chapter VII

(see Figure S1 in the supplementary material).

$$DALY_{pppy} = \left\{1 - \left(1 - P_{inf}(D)\right)^{n}\right\} \times R_{inf} \times DB \qquad \text{(Eq.3.3)}$$

$$P_{inf}(D) = {}_1F_2\left(\alpha, \frac{D(1-a)}{a}, \alpha + \beta; \frac{-a}{1-a}\right) \qquad \text{(Eq.3.4)}$$

Where,

$DALY_{pppy}$: DALYs per person per year

n : exposure frequency per year

R_{inf} : the probability of illness conditional on infection (0.8)

DB : disease burden (9.0×10^{-4})

$P_{inf}(D)$: the probability of infection

D : the number of consumed virus particles (copies/L)

a, α, β : fit parameters reported in Teunis et al. (2008)

Table 3.3 Exposure scenarios

Scenario	Purpose	Risk receptor	Route of exposure	Exposure frequency/ person/year	Volume for single exposure	Reduction in the environment	References
Scenario 1	Recreational impoundment	Swimmer	Accidental ingestion	40	100	No virus reduction	Tanaka et al., 1998
Scenario 2	Municipal irrigation	People involved	Ingestion	50	1	No virus reduction	NRMMC et al., 2006
Scenario 3	Garden irrigation	Residents involved	Routine ingestion	1	90	No virus reduction	NRMMC et al., 2006
			Accidental ingestion	100	1		
Scenario 4	Toilet flushing	Residents involved	Ingestion of sprays	1100	0.01	No virus reduction	NRMMC et al., 2006
Scenario 5	Crop irrigation	Consumer	Ingestion	140	1	Stop irrigation 2 weeks before harvest; 2 log virus was reduced by die-off during delivering to consumer; 1 log virus was removed by washing before eating	NRMMC et al., 2006; WHO., 20016

3.3 Result and discussion

3.3.1 ozonation

The virus removal performance by ozonation was evaluated using MS2 as surrogates. The ozone feed rate was 0.5 mgO_3/L/min. Figure 3.5 shows schematic diagram of the experimental procedure for ozonation. Experimental conditions for ozonation and the tested source water quality was listed in Table 3.4.

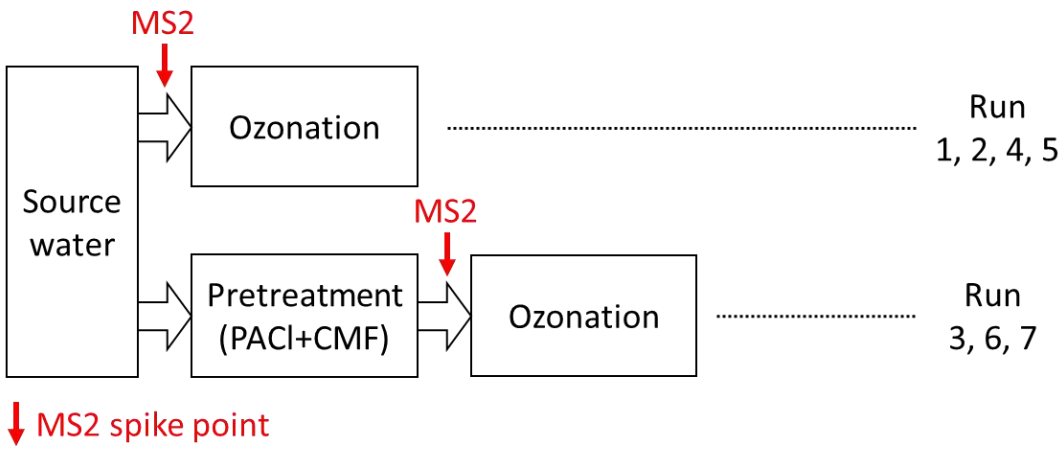

Figure 3.5 Schematic diagram of the experimental procedure for ozonation (PACl and CMF represent coagulation and ceramic membrane filtration, respectively)

Table 3.4 Experimental conditions for ozonation and the tested source water quality

Ozonation				
Run	Date	Source water	Pretreatment	Source water quality
				TOC (mg/L)
1	2013/07/22	SE	No	4.1
2	2014/01/28	SE	No	4.1
3	2014/01/28	SE	PACl(25mg/L)+CMF	3.3
4	2014/10/21	PE	No	56.3
5	2015/09/02	PE	No	38.8
6	2014/10/21	PE	PACl(150mg/L)+CMF	9.0
7	2015/09/02	PE	PACl(50mg/L)+CMF	10.7

Figure 3.6 shows MS2 removal rate by ozonation. The horizontal axis is O_3 consumption divided by initial TOC of source water. SE, CM permeate (SE), PE and CM permeate (PE) was used as source water.

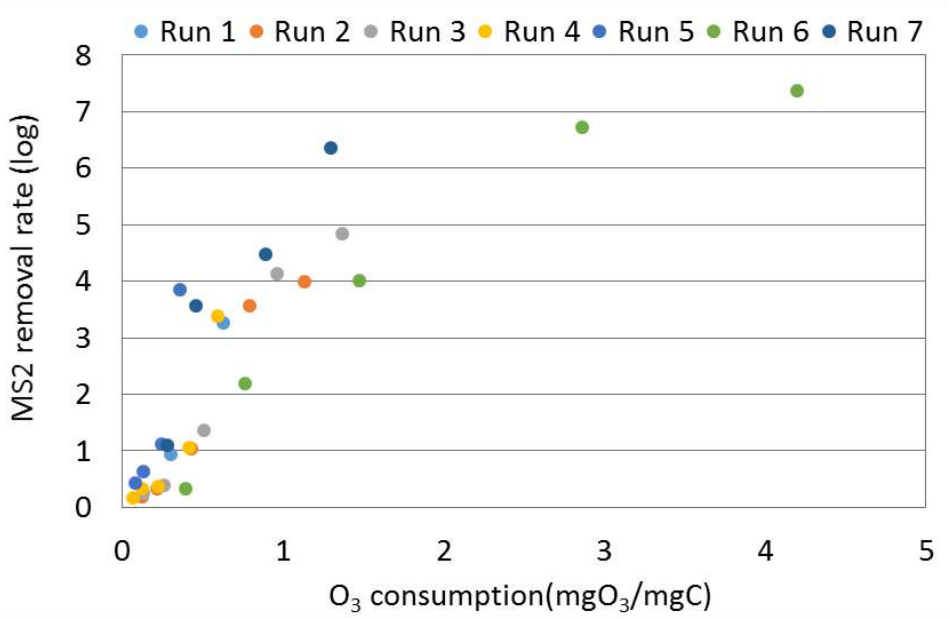

Figure 3.6 MS2 removal rate by ozonation

Even though each source water had different TOC values, as shown in Figure 3.6, similar removal rate was observed against same mgO_3/mgC. For instance, 4 log of MS2 removal rate was obtained under 1 mgO_3/mgC. It means that O_3 consumption (mgO_3/L) as much as their TOC value was required to obtain 4 log of removal rate in each source water. Also, the removal of TOC by CMF could reduce ozone dosage Especially, TOC value of PE was reduced to about 12 mg/L from 48 mg/L, and thus post-ozonation could reduce to a quarter of ozone dosage, compared with pre-ozonation. Therefore, it was able to save energy consumption for ozone generation through post-ozonation. These results were possible to provide ozone dosage required to achieve target virus removal rates against source water which have different TOC value. It could also contribute to determining ozone dosage when O_3&CMF process applied the other wastewater treatment plant.

3.3.2 Coagulation

3.3.2.1 MS2 removal by coagulation and sedimentation

Figure 3.7 shows schematic diagram of the experimental procedure for coagulation. Experimental conditions for coagulation and the tested source water quality was listed in Table 3.5. Ozonation was used as the pretreatment for coagulation and MS2 was spiked into source water after pretreatment.

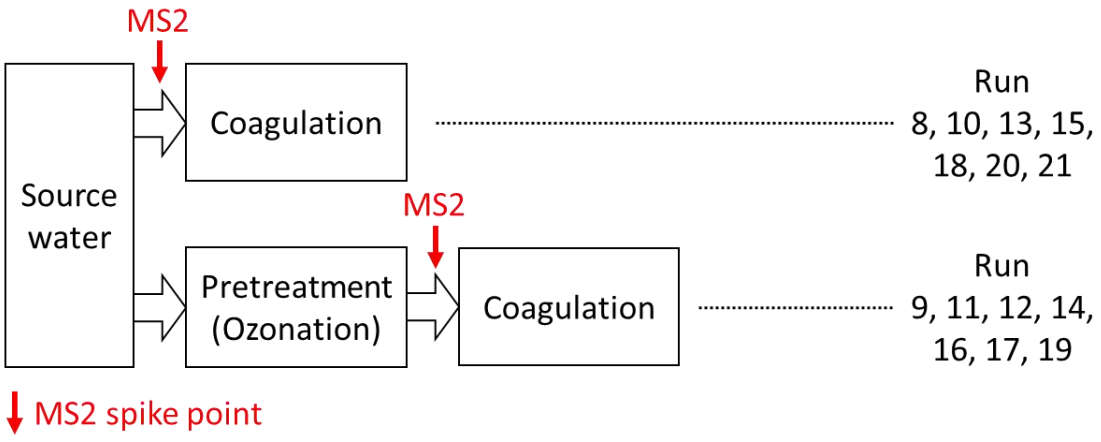

Figure 3.7 Schematic diagram of the experimental procedure for coagulation

Table 3.5 Experimental conditions for coagulation and the tested source water quality

Coagulation				
Run	Date	Source water	Pretreatment	Source water quality
				TOC (mg/L)
8	2013/01/10	SE	No	3.3
9	2013/01/10	SE	Ozonation (4mg/L)	3.4
10	2013/07/17	SE	No	5.2
11	2013/07/17	SE	Ozonation (2mg/L)	4.4
12	2013/07/17	SE	Ozonation (4mg/L)	5.8
13	2014/09/14	SE	No	3.3
14	2014/09/14	SE	Ozonation (4mg/L)	4.0
15	2013/11/27	PE	No	11.5
16	2013/11/27	PE	Ozonation (10mg/L)	15.9
17	2013/11/27	PE	Ozonation (20mg/L)	16.0
18	2014/01/15	PE	No	16.7
19	2014/01/15	PE	Ozonation (20mg/L)	17.6
20	2015/03/24	PE	No	31.1
21	2015/03/24	PE	No	53.1

Figure 3.8 described MS2 removal rate by CS. Coagulation experiment was triplicated for SE, and duplicated for PE. The value represents mean MS2 removal rate, and error bars indicate standard deviation.

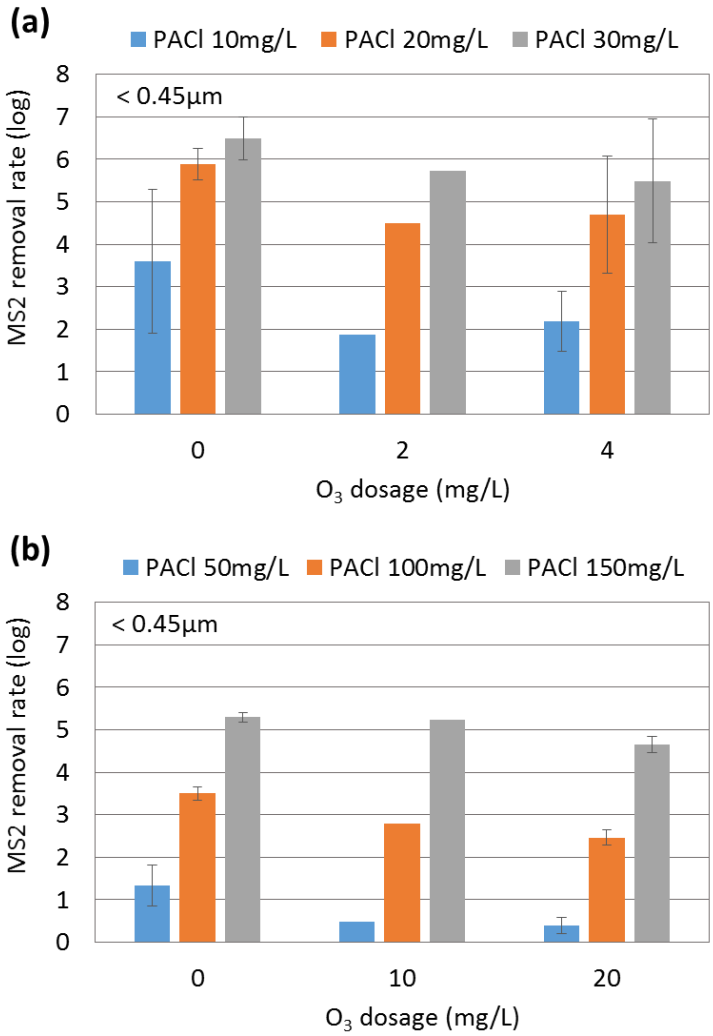

Figure 3.8 MS2 removal rate by CS in (a) SE and (b) PE
(MS2 removal rate by CS was calculated using MS2 concentration in liquid phase [<0.45 µm])

Under 10 mg/L of PACl dosage in SE, 3.6 log of MS2 removal rate was observed, and it increased to 6.5 log with increasing PACl dosage to 30 mg/L. In PE, 1.3 and 5.3 log of removal rate was obtained under 50 and 150 mg/L of PACl dosage, respectively. However, there were several reports that viruses could be inactivated by coagulants such as PACl (Matsui et al., 2003; Shirasaki et al., 2009; Matsushita et al., 2011, Kreißel et al., 2014). Therefore, these results included not only MS2 removal by CS but also MS2 inactivation by PACl. According to their reports, the specific spices in PACl such as dissolved aluminum polymers probably played a major role in virus inactivation during coagulation (Kreißel et al., 2014; Shirasaki et al., 2016). In addition, it was found that MS2 removal rate decreased by incorporating pre-ozonation. About 2 ~ 5.7 log and 0.4

~ 4.7 log of removal rate were observed in ozonated water, respectively, which were 1 ~ 2 lower than the removal rate in non-ozonated water (SE or PE). These differences between ozonated water and non-ozonated water became smaller with increasing PACl dosage. It would be discussed in detail in the next section.

Meanwhile, there was a report that MS2 removal by CS was related to DOC of source water (Lee, 2015). Thus, there is a possibility that MS2 removal by CS is affected by source water quality such as DOC. In the previous study, however, PE was not considered as a source water, and only SE was used. The huge amounts of particles in PE might influence on MS2 coagulation. For this reason, PACl dosage was normalized by TOC value of source water in this study, and the relationship between MS2 removal rate and PACl dosage/TOC was investigated. PACl dosage required to achieve target MS2 removal rate by CS is able to be estimated from this normalization result.

Figure 3.9 shows MS2 removal rate against PACl dosage divided by TOC value of source water. Circle and triangle represents MS2 removal rate in SE and PE, respectively.

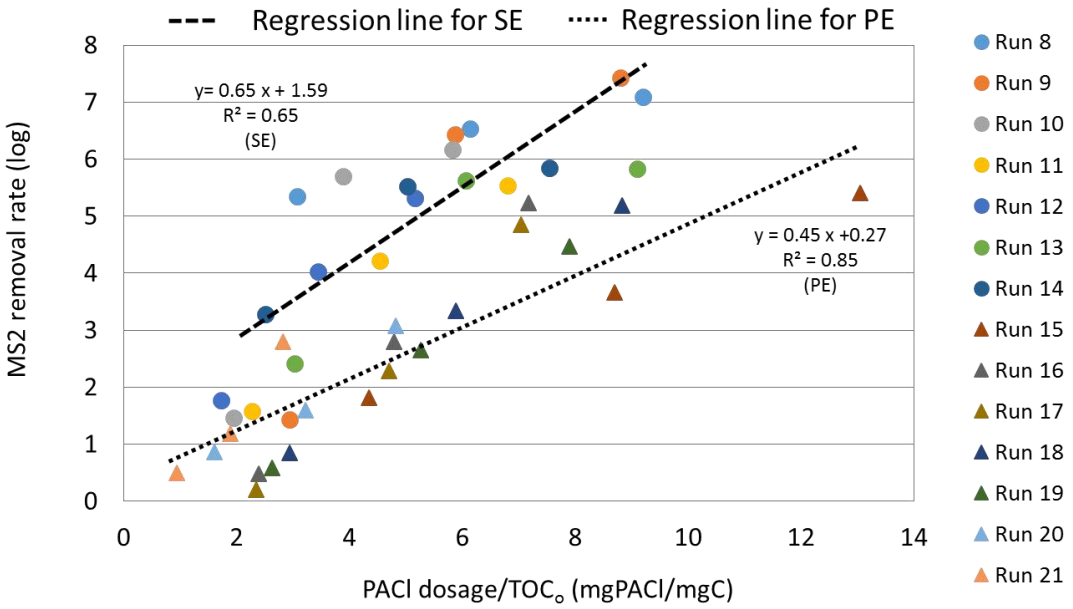

Figure 3.9 MS2 removal rate against PACl dosage/TOC

As a result, the relatively high correlation (R^2 = 0.65) was observed between MS2 removal rate and PACl dosage/TOC in SE, although there was a variation in MS2 removal rate under same PACl dosage/TOC. In case of PE, on the other hand, the much higher correlation (R^2 = 0.85) was observed compared to SE. However, there was 1 ~ 3 log difference between MS2 removal rate in SE and PE. Even though MS2 removal rate

might be normalized roughly by mgPACl/mgC in each SE and PE, there were an obvious difference between them. In case of coagulation, thus, it is necessary to consider the operational condition separately in SE and PE.

3.3.2.2 The effect of pre-ozonation on MS2 coagulation

As described in 3.3.2.1, a tendency that MS2 removal rate by CS decreased with increasing ozone dosage was observed in both SE and PE. Therefore, the effect of pre-ozonation on MS2 coagulation was investigated using SE as source water.

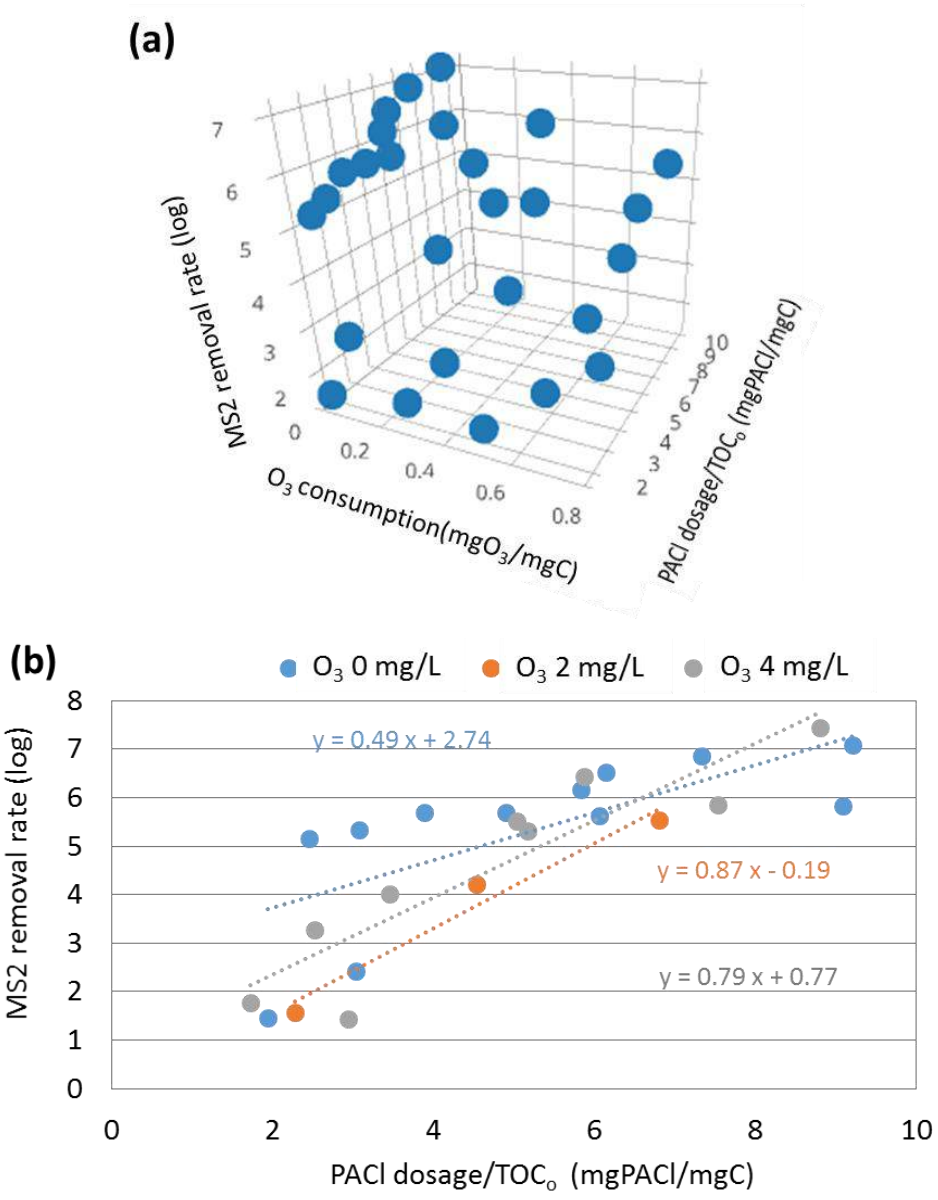

Figure 3.10 The effect of pre-ozonation on MS2 coagulation in SE represented by (a) 3D scatter plot and (b) scatter plot

It was found that MS2 removal rate decreased by pre-ozonation. As shown in Figure 3.10 (a), the lower MS2 removal rate was observed at higher mgO_3/mgC against the same PACl/TOC. In ozonated water, a much larger amount of PACl/TOC was required to obtain similar level with MS2 removal rate in SE (Figure 3.10 (b)). For example, PACl/TOC required to achieve 5 log of MS2 removal was 4.58 mgPACl/mgC in SE, whereas it was 5.97 and 5.35 mgPACl/mgC in 2 and 4 mg/L of ozonated water, respectively. However, there were no significant difference between the required PACl/TOC of ozonated water and non-ozonated water at higher than 6 log of removal rate (about 6.56 mgPACl/mgC). It corresponded to the above result that the difference in MS2 removal rate between ozonated water and non-ozonated water decreased with increasing PACl dosage.

These results indicated that pre-ozonation hinders MS2 coagulation, and the much larger amount of PACl dosage would be required to achieve target MS2 removal rate during coagulation. However, MS2 can be inactivated by DO_3 as mentioned in 3.3.1. The hindrance to MS2 coagulation might be attributed to the change of water quality by pre-ozonation. Ozonation could influence on changing the characteristics of organic matters. For instance, ozonation could converted hydrophobic organic matters into hydrophilic structure (Swietlik, 2004), and also degraded high molecular weight matter to low molecular weight matter (Edwards and Benjamin, 1992). Moreover, Li et al. (2009) reported that the relative polarity of ozonated water increased, and it was related to the formation of relatively polar ozonides, ketones, aldehydes, organic acids and functional groups during ozonation (Richardson et al., 1999, Glaze et al., 1991). This polarity and various ozonides formation during ozonation could influence surface charge of organic matters (Valdés, 2002). The surface charge played an important role in particle aggregation (Li et al., 2009; Chen et al., 2009). For this reason, we investigated the change of zeta potential by ozonation and coagulation.

Figure 3.11 shows the change of zeta potential during coagulation in SE. The horizontal axis and vertical axis indicate PACl dosage (mg/L) and zeta potential (mV), respectively. The value represent mean zeta potential, and error bars indicate the range. The legend represents ozone dosage of pre-ozonation

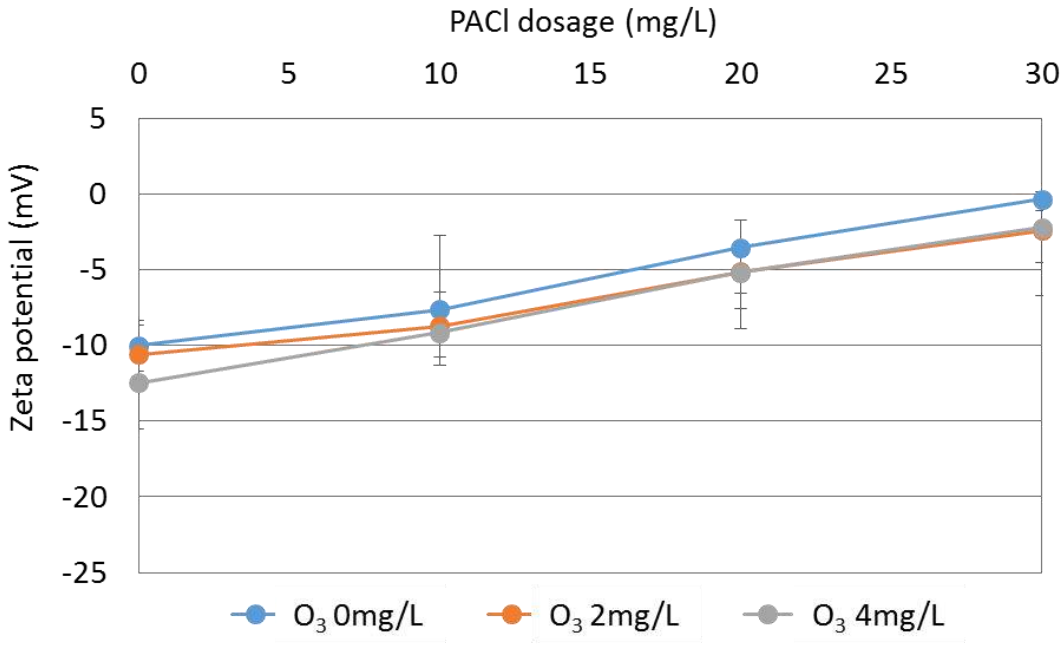

Figure 3.11 Zeta potential during coagulation in SE

Zeta potential was initial – 10mV in SE, and it increased more positive to – 7.6 and -0.4 mV with 10 and 30 mg-PACl/L, respectively. Zeta potential of ozonated water was initial -12.5mV, and it also increased positive to -9.1 and -2.2 with increasing PACl dosage. The zeta potential values in ozonated water were generally -2mV lower than that of SE, but the increase rate against PACl dosage was similar with SE. From the result of zeta potential, it was found that pre-ozonation negatively affects particles destabilization. Consequently, it was difficult to neutralize surface charge in ozonated water compared to non-ozonated water. Furthermore, zeta potential showed the tendency to increase with ozone dosage. Thus, the change of zeta potential during ozonation was investigated. Figure 3.12 shows the change of zeta potential with increasing ozone dosage. The value represents mean zeta potential, and error bars indicate standard deviation.

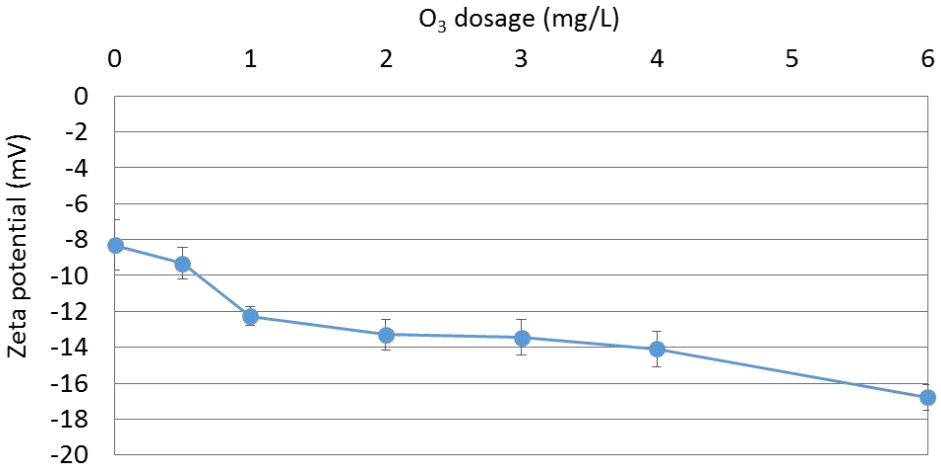

Figure 3.12 The change of zeta potential during ozonation in SE

The initial zeta potential was -8 mV in SE, but it negatively increased to -16 mV with increasing ozone dosage to 6mg/L. The hindrance of MS2 coagulation by pre-ozonation was attribute to the increases of negative charge, and this increase seems to be due to the change of polarity in ozonated water caused by the formation of ketones, aldehydes and functional groups.

3.2.2.3 MS2 removal by ozonation and coagulation

It was revealed that pre-ozonation hinders MS2 coagulation, but the MS2 inactivation by ozonation was excluded in above results. Therefore, it is necessary to evaluate MS2 removal rate including MS2 inactivation by ozonation. Figure 3.13 shows schematic diagram of the experimental procedure for ozonation and coagulation. Experimental conditions and the tested source water quality was listed in Table 3.6.

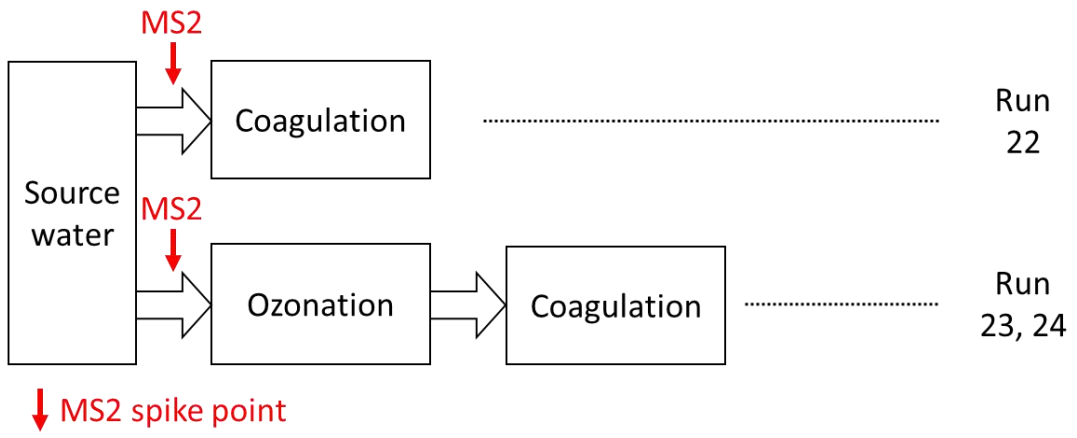

Figure 3.13 Schematic diagram of the experimental procedure for ozonation and coagulation

Table 3.6 Experimental conditions for ozonation and coagulation and the tested source water quality

Ozonation and coagulation				
Run	Date	Source water	Treatment	Source water quality
				TOC (mg/L)
22	2013/07/22	SE	Coagulation (25mg/L)	4.1
23	2013/07/22	SE	Ozonation (2mg/L)+ coagulation (10, 20 and 30 mg/L)	3.0
24	2013/07/22	SE	Ozonation (4mg/L)+ coagulation (10, 20 and 30 mg/L)	3.4

Figure 3.14 (a) shows the MS2 removal rate by both ozonation and CS. This experiment was conducted using SE spiked with MS2, and therefore the result contained MS2 removal by both ozonation and CS. The parenthesis in Figure 3.14 (a) represents MS2 removal rate by ozonation. Figure 3.14 (b) shows the total MS2 removal rate by ozonation and CS, in which the result of CS in 3.3.2.1 was aggregated with the removal by ozonation estimated from O_3 consumption (mgO_3/mgC) based on the result in 3.3.1.

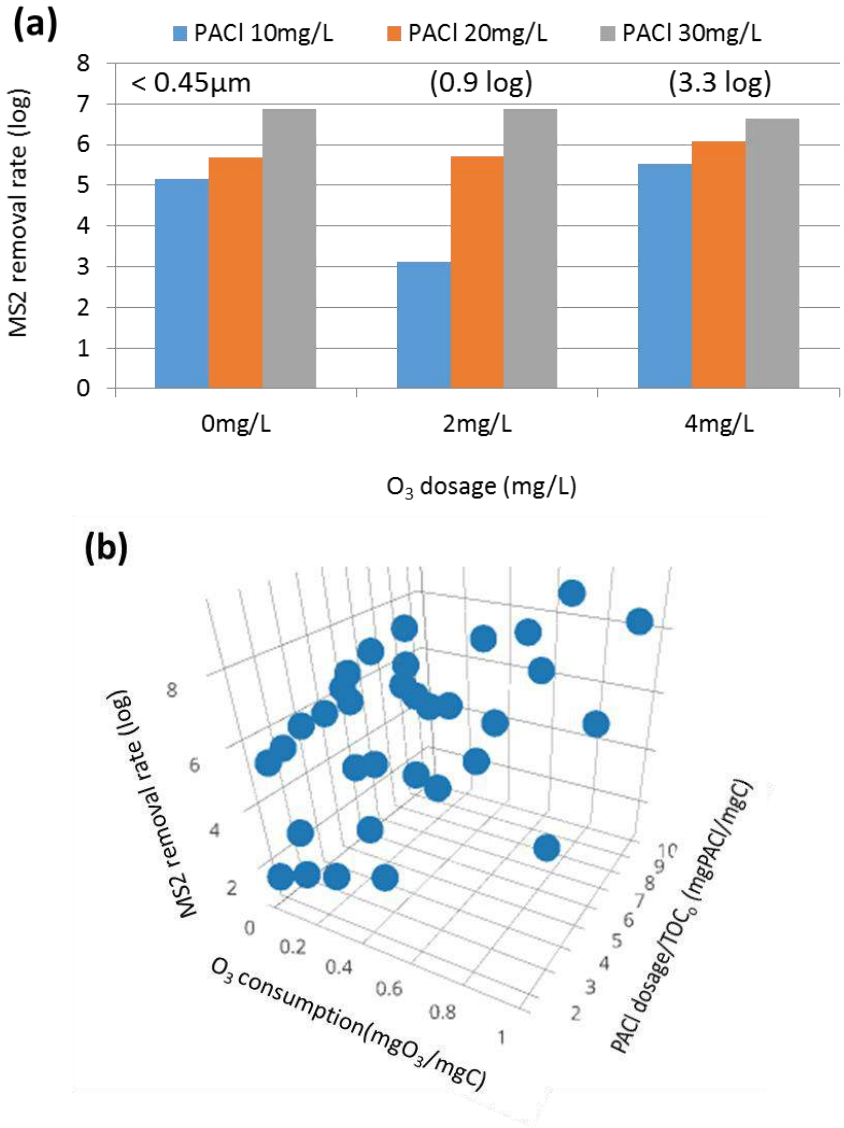

Figure 3.14 MS2 removal rate by ozoantion and CS in SE represented by (a) bar plot and (b) 3D scatter plot
(The parenthesis above bar plot represents MS2 removal rate by ozonation. MS2 removal rate by CS was calculated using MS2 concentration in liquid phase [<0.45 μm])

As shown in Figure 3.14 (a), 0.9 and 3.3 log of MS2 removal rate was obtained by 2 and 4 mg/L of ozonation, respectively. Despite of the hindrance to MS2 coagulation by pre-ozonation, as a result, the similar level of removal rate with SE (0mg/L of ozone dosage) was obtained under 4 mg/L of ozone dosage. The same tendency was able to be confirmed in Figure 3.14 (b). As opposed to above Figure 3.10 (a), MS2 removal rate increased with increasing ozone dosage, due to MS2 inactivation by ozonation. Thus,

pre-ozonation offset the hindrance of MS2 coagulation by its MS2 inactivation capability. However, the lower MS2 removal rate was able to be obtained at the relatively low ozone and PACl dosage. Indeed, the lower removal rate was observed under the condition of 2mg-O_3/L and 10 mg-PACl/L, compared to 0mg-O_3/L and 10 mg-PACl/L. Therefore, much caution is required to select the operation condition of O_3+PACl+CMF.

3.3.3 Selection of efficient treatment sequence for treating SE and PE based on the calculation of energy consumption

The estimated virus removal required in each scenario was summarized in Table 3.7. The virus removal required in each scenario was calculated in accordance with the method described in 3.2.6. In brief, the concentration of norovirus in source water was derived from the result in Chapter VII (see Figure S1 in the supplementary material). The target concentration of norovirus in reclaimed water was determined to satisfy the acceptable risk, defined as 10^{-6} DALY per person per year in drinking water set by WHO (WHO, 2004). The virus removal required in each scenario was calculated based on the observed virus concentration in source water and the calculated virus concentration in reclaimed water.

Table 3.7 Virus removal required in each scenario

Scenario	Target virus removal (log)	
	SE	PE
Scenario 1	8.3	9.3
Scenario 2	6.4	7.4
Scenario 3	6.7	7.7
Scenario 4	5.8	6.8
Scenario 5	4.0	5.0

In O_3&CMF process, total energy consumption was decided depending on each operational condition in ozonation and PACl+CMF. Therefore, several cases could be assumed depending on the proportion of virus removal in each ozonation and PACl+CMF. In this study, each three unit processes were assumed in O_3+PACl+CMF and PACl+CMF+O_3.

The assumption was summarized in Table 3.8. Process 1, 2 and 3 represent O_3+PACl+CMF for treating SE or PE, and process 4, 5 and 6 represent PACl+CMF+O_3 for treating SE or PE. It was assumed that 1, 2 and 4 log of virus removal rate was

obtained during pre-ozonation in process 1, 2 and 3 (or during PACl+CMF in process 4, 5 and 6) for treating SE, respectively. The remains of virus removal rate to achieve target virus removal required to each scenario was obtained by PACl+CMF in process 1, 2 and 3 (or by post-ozonation in process 4, 5 and 6). Meanwhile, process 1 ~ 6 for treating PE was also assumed in the same way as process 1 ~ 6 for treating SE. Only the assumed virus removal rate to obtain during pretreatment (pre-ozonation in process 1, 2 and 3; PACl+CMF in process 4, 5 and 6) was changed to 1, 3 and 5 log.

The result of energy consumption was summarized in Table S1 and S2 (see Table S1 and S2 in the supplementary material).

Table 3.8 The assumption on the proportion of virus removal in O_3+PACl+CMF and PACl+CMF+O_3

Target virus removal (log) (SE)					
	O_3+PACl+CMF			PACl+CMF+O_3	
Process	Pre-ozonation	PACl+CMF	Process	PACl+CMF	Post-ozonation
Process 1	1	r[a]	Process 4	1	r[a]
Process 2	2	r[a]	Process 5	2	r[a]
Process 3	4	r[a]	Process 6	4	r[a]
Target virus removal (log) (PE)					
	O_3+PACl+CMF			PACl+CMF+O_3	
Process	Pre-ozonation	PACl+CMF	Process	PACl+CMF	Post-ozonation
Process 1	1	r[a]	Process 4	1	r[a]
Process 2	3	r[a]	Process 5	3	r[a]
Process 3	5	r[a]	Process 6	5	r[a]

[a]The remains (except for the achieved virus removal in pretreatment) to achieve target virus removal required for each scenario

Figure 3.14 shows the energy consumption of O_3&CMF process (O_3+PACl+CMF and PACl+CMF+O_3) for treating SE to achieve virus removal required for each scenario.

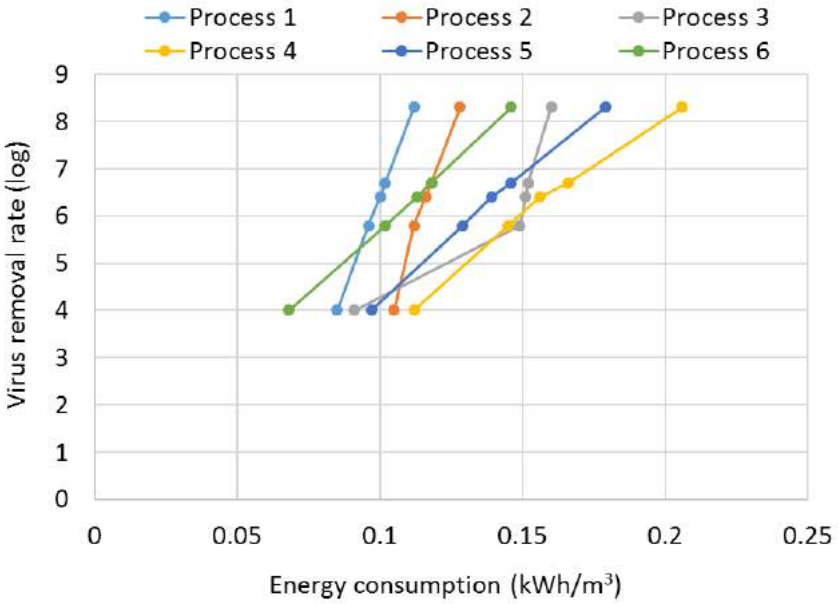

Figure 3.15 Energy consumption of O_3&CMF process for treating SE

The energy consumption of process 1, 2 and 3 (O_3+PACl+CMF) ranged in 0.09 ~ 0.11, 0.11 ~ 0.13 and 0.09 ~ 0.16 kWh/m^3, and process 4, 5 and 6 (PACl+CMF+O_3) ranged in 0.11 ~ 0.21, 0.10 ~ 0.18 and 0.07 ~ 0.15 kWh/m^3, respectively. In process 3, it was possible to achieve target virus removal required in scenario 5 (4 log) only by ozonation, and therefore energy consumption of PACl+CMF was not included in this case. Because of high energy consumption in ozonation, much lower energy was consumed with lower ozone dosage. In case of scenario 5, which required relatively low virus removal, however, target virus removal was achieved by only ozonation with low energy consumption. Also, O_3+PACl+CMF was more economical process in case that high virus removal was required such as scenario 1. In addition, the difference in energy consumption of O_3+PACl+CMF and PACl+CMF+O_3 was a maximum level of 0.03 kWh/m^3, and it is possible to be compensated by the reduction of chemical cost for CEB because pre-ozonation can mitigate membrane fouling. Thus, it was expected that reclaimed water which has more various purposes can be produced economically by appropriate O_3+PACl+CMF in case of the treatment for SE.

Consequently, O_3+PACl+CMF was selected as treatment process for SE, and continuous operation was conducted in Chapter IV.

Figure 3.15 shows the energy consumption of O_3&CMF process (O_3+PACl+CMF and PACl+CMF+O_3) for treating PE to achieve virus removal required for each scenario.

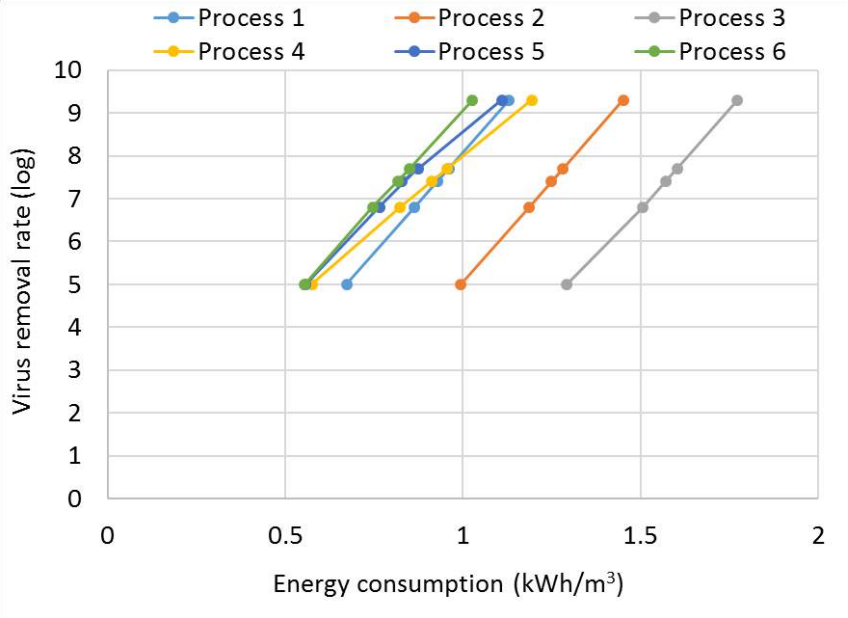

Figure 3.16 Energy consumption of O_3&CMF process for treating PE

In O_3&CMF process for treating PE, the energy consumption of process 1,2 and 3 (O_3+PACl+CMF) ranged in 0.67 ~ 1.13, 0.99 ~1.45 and 1.29 ~ 1.77 kWh/m^3, and process 4,5 and 6 (PACl+CMF+O_3) ranged in 0.58 ~ 1.19, 0.56 ~ 1.11 and 0.56 ~ 1.02 kWh/m^3, respectively. Generally, the energy consumption of PACl+CMF+O_3 was at least 0.1 ~ 0.2 kWh/m^3 lower than that of O_3+PACl+CMF. As similar with the result in SE, in addition, it was much economical to set ozone dosage as low as possible in both case of O3+PACl+CMF and PACl+CMF+O3. In O3+PACl+CMF, especially, the energy consumption largely increased with increasing ozone dosage. Therefore, it seems that it is difficult to compensate energy consumption on pre-ozonation by the reduction of chemical cost of CEB because relatively high ozone dosage was required to be effective in mitigating membrane fouling.

On the basis of these results, it was expected that PACl+CMF+O_3 was much economical process for treating PE than O_3+PACl+CMF. Thus, PACl+CMF+O_3 was selected as treatment process for treating PE, and the continuous operation was conducted in following chapter IV.

3.4 Conclusions

In this chapter, virus removal performance of both ozonation and coagulation was evaluated, and moreover the effect of pretreatment on subsequent treatment was investigated, considering process sequence in O_3+PACl+CMF and PACl+CMF+O_3. In

addition, the energy consumption of O_3&CMF process to achieve target virus removal required for each scenario was calculated. On the basis of calculation results, the efficient process sequence of O_3&CMF process for treating SE and PE was selected.

The following conclusions can be drawn:

1. In ozonation, similar MS2 removal rate was observed under same mgO_3/mgC, even though source water had a different TOC or DOC value each other. The reduction of TOC by CMF was contributed to the decreases of the required ozone dosage. It could also contribute to determining the condition ozone dosage when O_3&CMF process applied the other wastewater treatment plant.

2. In coagulation, 3.6 ~ 6.5 log of MS2 removal rate was obtained under 10 ~ 30 mg/L of PACl dosage in SE, and 1.3 ~ 5.3 log of removal rate was observed under 50 ~ 150 mg/L of PACl dosage in PE. MS2 removal rate might be normalized roughly by mgPACl/mgC, but it needs to be conducted in SE and PE separately.

3. MS2 removal rate by CS tended to decrease by pre-ozonation. In ozonated water, the much larger amount of PACl/TOC was required to obtain similar level with MS2 removal rate in SE.

4. The hindrance of MS2 coagulation by pre-ozonation was attribute to the increases of negative charge, and this increase seems to be due to the change of polarity in ozonated water. However, the hindrance of MS2 coagulation was compensated by MS2 inactivation capability of pre-ozonation.

5. From the result of the calculation of energy consumption, it was expected that O_3+PACl+CMF and PACl+CMF+O_3 was efficient process for treating SE and PE, respectively.

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Chapter Ⅳ

Operational and virus removal performance of ozonation and ceramic membrane filtration combination process

4.1 Introduction

In previous chapter, the efficient process sequence in ozonation and ceramic membrane filtration combination process (O_3&CMF process) was investigated. Ozonation followed by ceramic membrane filtration (O_3+PACl+CMF) and ceramic membrane filtration followed by ozonation (PACl+CMF+O_3) was selected for treating secondary effluent (SE) and primary effluent (PE).

As mentioned before, it was expected that O_3+PACl+CMF has diverse advantages on removal and operational performance compared with other conventional membrane filtration processes. While many researches have been reported that membrane fouling was mitigated by incorporating ozonation as pretreatment for membrane filtration (Kim et al., 2008; Park et al., 2010; Zhu et al., 2010; 2012; Van Geluwe et al., 2011; Zhang et al., 2013; Fan et al., 2014; Cheng et al., 2016; Wei et al., 2016), there are few researches regarding long−term operational performance of O_3&CMF process that both ozonation and coagulation were used as pretreatment in sequence. According to a previous research, the ceramic membrane demonstrated stable performance for approximately 680 hours at a flux of 4 m/d with pretreatment using ozoantion (4 mg/L) and coagulation (1 mg-Al/L, polyaluminium chloride [PACl]) (Lehman et al., 2009). However, long-term operation of O_3&CMF process was conducted under the one operational condition and without chemical enhanced backwashing (CEB). CEB is an indispensable procedure when membrane fouling was aggravated irretrievably. Moreover, they have not provided

any information with regard to virus removal performance of O_3&CMF process, which directly linked with health risk of reclaimed water users. The development of efficient O_3&CMF process through the evaluation of both removal and operational performance is needed in order to supply stable and hygienically safe reclaimed water from the viewpoint of both quantity and quality of that. In this chapter, therefore, the operational performance of O_3&CMF process was evaluated through long-term continuous operation with CEB, and a virus removal performance was also evaluated using bacteriophage MS2 (MS2) as a model virus.

In addition, there are only a few studies on application of membrane filtration for treatment of primary effluent despite potential benefits such as energy saving for aeration in activated sludge (Abdessemed et al., 1999; 2002; Ravazzini et al., 2005; Diaz et al., 2012; Jin et al., 2015). Ravazzini et al. (2005) reported that the average flux was 160 $L/h/m^2$ under constant transmembrane pressure (TMP) (0.3 bar) operation of ultrafiltration with a filtration cycle of 10 min and 1min of backflush at cross flow velocity of 2 m/s. However, these studies evaluated the operation performance of membrane filtration for treating PE through a short-term operation, and also did not consider the treatment process including post-ozonation which could inactivate virus but also effectively remove odors and colors.

Thus, both operational and virus removal performance of O_3&CMF process for treatment of primary effluent was also evaluated in a similar manner as the case of secondary effluent.

4.2 Materials and Methods

4.2.1 Water quality items

Water quality items were analyzed in accordance with the method described in 3.2.1.

4.2.2 Model virus

MS2 was selected as model virus to evaluate virus removal performance of O_3&CMF process. The preparation of MS2 stock suspension and MS2 analysis was conducted according to methods described in 3.2.1.

4.2.3 Treatment experiment for secondary effluent

O_3&CMF process for treating SE was conducted continuously in order of ozonation, coagulation and ceramic membrane filtration.

4.2.3.1 Experimental setup

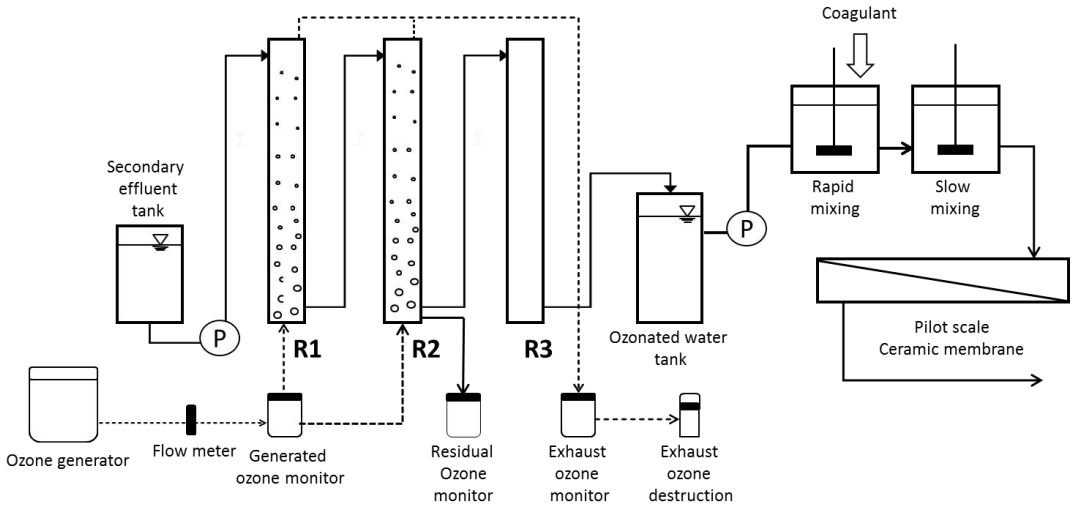


Figure 4.1 Experimental setup for secondary effluent treatment

Table 4.1 Characteristics of the pilot scale membrane

Monolith ceramic membrane (Internal pressure)	
Membrane module size	ϕ 30mm × 1,000mm
Channel Number	55 channels
Effective Area	0.42m^2
Pore Size	0.1μm

Figure 4.1 shows the experimental setup of pilot scale ozonation and ceramic membrane.

Secondary effluent, which filled in a source water tank was flowed into an ozone reactor with a flow rate of 7L/min. The turbidity of secondary effluent in the source water tank was monitored by a turbidity meter. Three bench scale ozone reactors were used for ozonation, and each ozone reactor has a volume of 35L and a retention time of 5min. The ozone gas was generated using an ozone generator with dielectric barrier discharge (FZH-12, Fuji electronics). The generated ozone was injected into both first (R1) and second ozone reactor (R2). Third ozone reactor (R3) was used for only retention. The

flow rate of generated ozone was controlled by flow meter (SUS-316, Flow-Cell Co.). The concentrations of generated ozone, exhaust ozone and residual ozone were measured by ozone monitors (EG-600, Ebara, OZ-20, OZ-30, DKK-Toa). The ozone after passing ozone monitor was exhausted through the ozone destruction units. Ozonated water was collected in a tank, and then flowed to coagulation tanks at constant flow rate (2L/min) by a magnetic drive pump (MD-15RN、IWAKI). Two coagulation tanks, which have a volume of 8L each, were prepared for rapid (G=680.3 s^{-1}) and slow mixing (G=130.9 s^{-1}), respectively. It was expected to improve the coagulation efficiency by connecting two coagulation tanks in series, and also mitigate irreversible membrane fouling. PACl (10 ~ 11% Al_2O_3, Takasugi pharmaceutical) was used as coagulant, and injected in the rapid mixing tank at a constant dose rate (25mg-PAC/L) by a peristaltic pump (SJ-1211、ATTO). After coagulation, coagulated water fed into ceramic membrane filtration. The filtration was operated at the constant flux 4m/d (1.1L/min) in a dead-end mode and continued for 1h. At the end of each filtration cycle, the ceramic membrane was backwashed at a pressure of 300kPa with the filtrate for 30s, and was followed by an air blow with compressed air at a pressure of 300kPa. CEB was conducted using both 10% of sulfuric acid (10 mg/L) and sodium hypochlorite (500 mg/L) when TMP was higher than 60kPa even though CM was backwashed. The ceramic membrane was soaked in filtrated water which contains sulfuric acid for 30min, and then the same procedure was repeated once again using sodium hypochlorite.

4.2.3.2 Experimental methods for MS2 spike test

The continuous operation of O_3&CMF process was stopped temporarily when MS2 spike experiments were conducted. The MS2 spike experiments were conducted separately at ozonation part(O_3)/coagulation and ceramic membrane filtration part (PACl+CMF), because there was a possibility that MS2 would not be detected in ceramic membrane filtrate if secondary effluent spiked with MS2 was treated continuously. In ozonation part, therefore, MS2 was spiked in source water tank filled with secondary effluent. In coagulation and ceramic membrane filtration part, the other ozonated water tank filled with 200L of ozonated water was prepared for MS2 spike experiments. After residual ozone of ozonated water in the tank was extinguished, MS2 was spiked. MS2 suspensions was added to each tank at approximately 10^6 ~ 10^7PFU/ml. All of samples was collected considering hydraulic retention time.

4.2.4 Primary effluent experiment

O_3&CMF process for treating PE was conducted in order of coagulation, ceramic membrane filtration and ozonation. Coagulation and ceramic membrane filtration was conducted continuously, whereas ozonation was performed using the semi-batch ozone reactor separately.

The order of ozonation was changed to final treatment of O_3&CMF process because it was revealed that O_3+PACl+CMF was less efficient in terms of membrane fouling mitigation than PACl+CMF+O_3 when primary effluent was used as the source water in Chapter III. Huge amounts of energy were needed to mitigate membrane fouling due to plenty of organic matters in primary effluent. Furthermore, ozonation efficiency could be improved by removal of suspended solid (SS) or particle matters during ceramic membrane filtration. Therefore, it was more efficient that ozonation was conducted as posttreatment of ceramic membrane filtration than pretreatment if energy consumption were the same.

4.2.4.1 Experimental setup of coagulation and ceramic membrane filtration

PACl+CMF experiment was conducted using the experimental setup described in 3.2.4.3

4.2.4.2 Experimental setup of ozoantion

Ozonation experiment was conducted using the experimental setup described in 3.2.1

4.2.4.3 Experimental methods for MS2 spike test

Experimental methods for MS2 spike test was similar with 4.2.3.2, but the order of treatment was different. In coagulation and ceramic membrane filtration part, MS2 was spiked into the source water tank filled with 50L of primary effluent, and then samples of ceramic membrane filtration were collected. In ozonation part, the collected ceramic membrane filtrate spiked with MS2 was subjected to ozonation using the semi-batch reactor describe in 3.2.1. Initial MS2 concentration of both primary effluent and ceramic membrane filtrate, was approximately 10^6~10^7PFU/ml. All of samples was collected considering hydraulic retention time.

4.2.5 Fouling resistance analysis

The influence of pre-ozonation on ceramic membrane fouling was investigated using the resistance in series model (Gésan-Guisiou et al., 1999; Lin et al., 2009; Wei et al., 2016). The resistance of reversible and irreversible fouling was calculated in accordance with Darcy's law, as shown in Eq. 4.1 and Eq. 4.2.

$$R_{\mathrm{t}} = \frac{\mathrm{V}d}{\mathrm{A}d\mathrm{t}} = \frac{\Delta \mathrm{P}}{\mu J} \quad \text{(Eq.4.1)}$$

$$R_{\mathrm{t}} = R_{\mathrm{m}} + R_f = R_{\mathrm{m}} + R_r + R_{ir} \quad \text{(Eq.4.2)}$$

Where,

J : Permeate flux ($m^3/m^2/s$ or m/s)

A : Effective membrane surface (m^2)

V : Permeate volume (m^3)

ΔP : TMP (Pa, $kg/m/s^2$)

μ : Viscosity of water (kg/m/s or cP, 0.8937x10-3 kg/m/s for water at 25 ℃)

R_t : Total membrane resistance (m^{-1})

R_m : Intrinsic membrane resistance (m^{-1})

R_f : Fouling resistance (m^{-1})

R_r : Reversible fouling resistance (m^{-1})

R_{ir} : Irreversible fouling resistance (m^{-1})

4.2.6 Energy consumption calculation

Energy consumption for oznation and coagulation was calculated using the method described in 3.2.5.1 and 3.2.5.2, respectively. The electricity consumption during CMF operation was calculated in 3.2.5.3. In this chapter, in addition, energy consumption for CMF operation was recalculated including chemical cost consumed for CEB. It was investigated that the effect of pre-ozonation on saving energy consumption for CEB.

In this study, it was assumed that 20 module of full scale ceramic membrane, which has $50m^2$ of an effective area, are used for treating 4000 m^3/d. CEB was conducted using both 10% H_2SO_4 (10 mg/L) and NaClO (500 mg/L). According to our experience of CEB, 4 ml of H_2SO_4 and 10 ml of NaClO was used for bench scale ceramic membrane (an

effective are was 0.42 m^2). Thus, the amount of H_2SO_4 and NaClO required for full scale ceramic membrane was 17.5 and 25.7 kg, respectively. Also, these chemicals were neutralized by NaOH and $Na_2S_2O_3$, respectively, before discharging them after CEB. The amount of NaOH and $Na_2S_2O_3$ required for neutralization was 14.3 and 21.4 kg, respectively. In basis of these assumption, the energy consumption for CEB was calculated using carbon footprints of each chemicals and electricity generation. The carbon footprint for the production of H_2SO_4, NaClO, NaOH and $Na_2S_2O_3$ were 0.087, 0.321, 0.938 and 2.980 $kgCO_2/kg$, respectively (Editing committee of LCA practical guide, 1998; JEMAI, 2012). The carbon footprint of electricity generation was 0.555 $kgCO_2/kWh$ (Editing committee of LCA practical guide, 1998). Consequently, energy consumption was 156.409 kWh/CEB, and it would be divided by the volume of treated water based on CEB interval.

4.3 Results and discussion

4.3.1 Performance of O_3&CMF process for treating secondary effluent

4.3.1.1 Water quality items

Water quality of SE, pre-ozonated water and ceramic membrane permeates are summarized in Table 4.3.

Table 4.2 Water quality in O_3+PACl+CMF process (average values)

Date	Ozone dosage (mg/L)	Samples	TOC (mg/L)	DOC (mg/L)	UV_{254} (cm^{-1})	Turbidity (NTU)
2014/01/23~2014/01/29	0	SE	4.6	4.0	0.067	3.9
		Ozonated water	4.6	4.0	0.067	3.9
		CM permeate	3.3	3.3	0.056	N.D
2014/01/12~2014/01/22	2	SE	4.6	4.0	0.065	2.4
		Ozonated water	4.5	3.8	0.039	1.5
		CM permeate	3.5	3.5	0.030	N.D
2013/12/11~2014/01/05	4	SE	4.7	4.5	0.061	2.9
		Ozonated water	4.3	4.3	0.026	1.5
		CM permeate	3.8	4.2	0.020	N.D
2015/12/04~2016/02/01	6	SE	3.9	4.3	0.059	2.6
		Ozonated water	4.2	4.6	0.033	1.7
		CM permeate	4.1	3.8	0.025	N.D

N.D : Not detected

Pre-ozonation could remove UV_{254} effectively, but there were no significant change regarding TOC and DOC. After PAC+CM, DOC content only remained, TOC values were

similar with DOC in CM permeates, because particulate organic matters was removed. The removal rates of TOC, DOC and UV_{254} were 24 ~ 39 %, 7 ~ 18 % and 16 ~ 67 %, respectively, by O_3+PACl+CMF. Turbidity was 2.4 ~ 3.9 NTU in SE, and totally eliminated by PACl+CMF.

4.3.1.2 Virus removal performance

Figure 4.3 shows MS2 removal rate in (a) pre-ozonation and (b) PACl(25mg/L)+CMF. The value in Figure 4.3 (b) indicates mean MS2 removal rate, and error bar represents standard deviation. MS2 spike test in PACl+CMF was triplicated at each O_3 dosage.

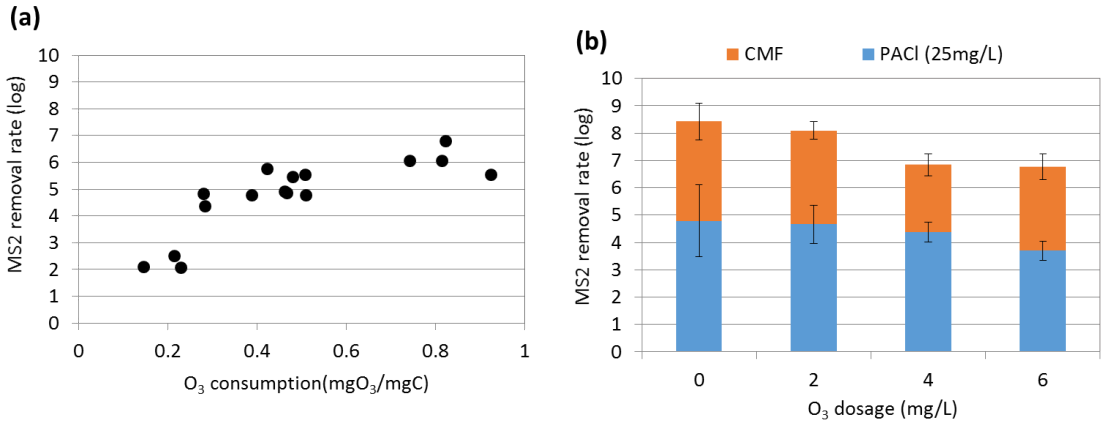


Figure 4.2 MS2 removal rate in (a) pre-ozonation and (b) PACl(25mg/L)+CMF

In pre-ozonation, MS2 removal rate was 2 log at 0.2 mgO3/mgC, and it increased to 5 log with increasing O3 consumption to 0.4 ~ 0.5 mgO3/mgC. However, there was no significant increase at higher than 0.5 mgO3/mgC. The removal rate increased by only 1 log between 0.5 and 0.8 mgO3/mgC, which seemed to be tail off phenomenon. It has been documented that particles can protect bacteria and viruses from chemical disinfectants or UV disinfection (Ormeci and Linden, 2002; Templeton et al., 2005; Shin et al., 2008). Therefore, virus aggregated each other or associated with particles is difficult to be inactivated through the ozonation, and causes the tail off phenomenon. The small increases in removal rate despite of increasing ozone dosage might be explained by the particle shielding effect.

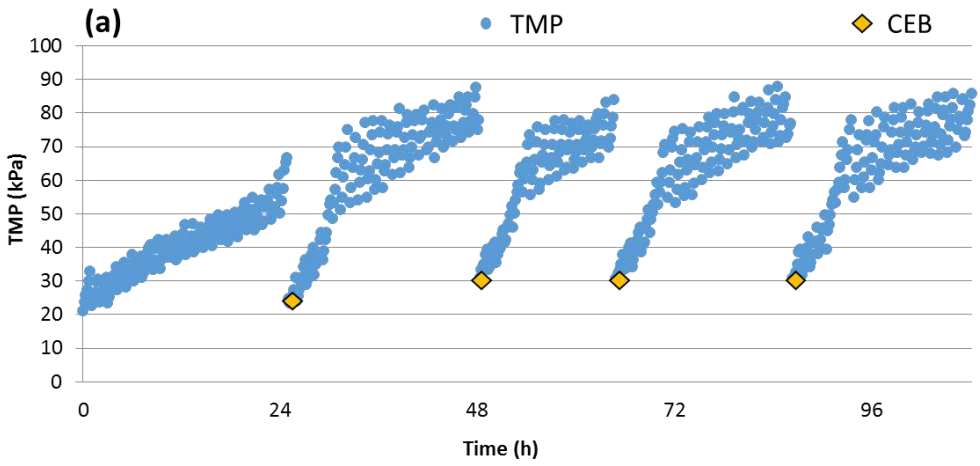
MS2 removal rate of 6 to 8 log was observed in PACl+CMF, and it was 1 to 3 log higher than that of coagulation and sedimentation in 3.3.2.1, indicating that MS2 was more effectively removed by PACl+CMF. Furthermore, the removal rate by CMF increased to

2.5 to 3.6 log with incorporating coagulation as pretreatment, while 0.2 log of removal rate was obtained by CMF without coagulation (see Figure S2 in the supplementary material). According to Huang et al. (2012), large effluent organic matter (EfOM), regarded as membrane foulants, can contribute to virus removal during membrane filtration. They reported that MS2 removal decreases by approximately 1.4 log, caused by the removal of large EfOM prior to membrane filtration. They elucidated that the formation of cake layer and pore blocking by large EfOM might interrupt virus which pass through the membrane, or absorb it. Thus, the increase of MS2 removal in CMF seemed to due to the formation of cake layer and pore blocking caused by much larger particle size in coagulated water compared to SE (non-coagulated water).

In addition, the removal rate tended to decrease with increasing ozone dosage, caused by the hindrance of MS2 coagulation by pre-ozonation as described in 3.2.2.2. Indeed, the removal rate by coagulation decreased from 4.8 to 3.7 with increasing ozone dosage from 0 to 6 mg/L. This decrease of removal rate in coagulation affected the removal performance in CMF, and the removal rate by PACl+CMF also decreased from 8.4 to 6.8. However, ozonation could inactivate MS2 effectively, and as a result the overall MS2 removal rate in O_3+PACl+CMF was higher than 12 log (see Figure S3 in the supplementary material).

4.3.1.2 Operational performance

Figure 4.4 shows TMP trend in the continuous operation of O_3+PACl+CMF for treating SE.



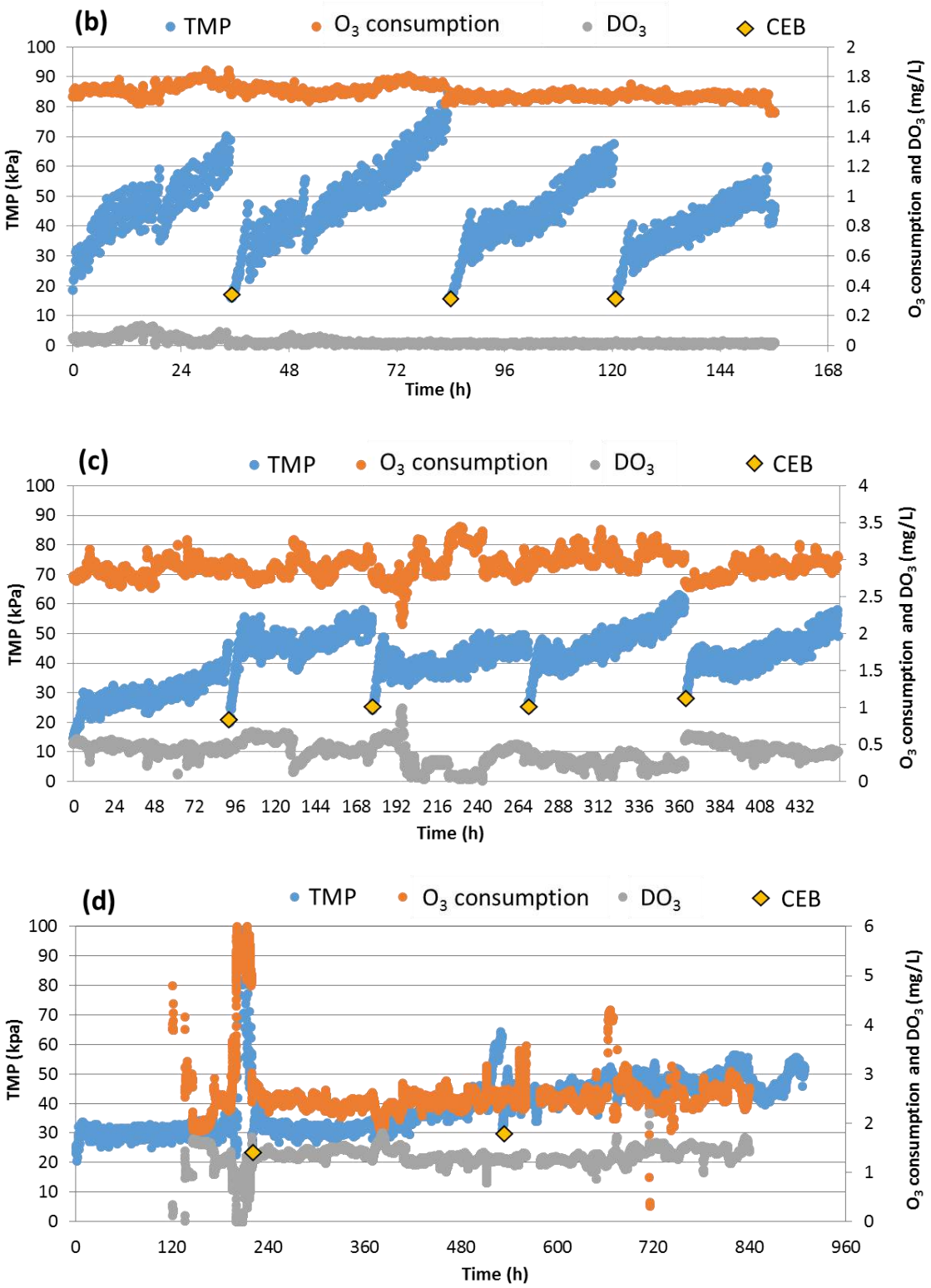


Figure 4.3 Operational performance under ozone dosage of (a) 0mg/L, (b) 2mg/L, (c) 4mg/L and (d) 6mg/L (PACl dosage : 25 mg/L; Flux : 4 m/d)

O_3+PACl+CMF under the condition of 0 mg-O_3/L, 25mg-PAC/L and Flux 4m/d was operated for 108 h (Figure 4.4 (a)). The turbidity of SE ranged from 2 to 4.5 NTU, and CEB was conducted 4 times during the operation period. A TMP increased gradually and then reached up to 60 kPa after 24 h. The 1st CEB was conducted at the time of 24 h. Although the TMP recovered to about 20kPa by CEB, it increased rapidly to 75 kPa within 24 h. Consequently, CEB was needed once a day for stable operation.

The operation of O_3+PACl+CMF was conducted for 156 h under 2 mg-O_3/L, 25mg-PACl/L and Flux 4m/d (Figure 4.4 (b)). There were 3 times of CEB during the operation period. The turbidity of SE was 0.7 to 3.2 NTU. O_3 consumption was 1.6 to 1.8 mg/L and DO_3 was below than 0.1 mg/L. These results of O_3 consumption and DO_3 suggested that most of the ozone reacted with organic matters in SE. TMP was 33 to 50 kPa for the first 24 h, but it increased from 40 to 70 kPa for the next 20 h. A tendency that TMP increase rate, the slope of TMP against time, increased at the some point was observed. It took about 34 h until 1st CEB, and the time required to reach the TMP of 60 kPa was also 36 to 48 h following 2nd and 3rd CEB. Therefore, the average CEB interval was estimated as about 48 h.

The O_3+PACl+CMF was operated with the condition of 4 mg-O_3/L, 25mg-PAC/L and Flux 4m/d (Figure 4.4 (c)). CEB conducted four times in 454 h of operation. The turbidity of SE was in range of 0.8 to 4.0 NTU. O_3 consumption and DO_3 was 2.7 to 3.3 mg/L and 0.1 to 0.6 mg/L, respectively. A little DO_3 which could not react with organic matters was detected as residual ozone. TMP was generally stable ranging from 40 to 60 kPa in operation period, and the CEB interval was approximately 90 h. Also, TMP increase rate increased at the some point, as similar with above results. According to Zhu et al. (2012), there appears to be a turning point in the curve of irreversible fouling. Irreversible fouling, which increased slowly before reaching the turning point, jumped after the turning point to a maximum level in a short time. They elucidated that it was caused by the development of “homogeneously-distributed pore constriction”, not “partial pore blockage”. Therefore, the turning point occurred when the majority of membrane pore evenly constricted or be clogged by the colloidal particles. It was presumed that the change of TMP increase rate, observed in this study, was also caused by similar reasons with the previous report. Furthermore, it was also found that the initial TMP after CEB gradually increased with repeated CEB. The initial TMP after 1st, 2nd and 4th CEB was 20, 24, 28 kPa, respectively. It indicated that fouled ceramic membrane does not restore completely only by CEB, and therefore the periodical cleaning in place (CIP) was required for stable long-term operation of O_3+PACl+CMF.

The operation of O_3+PACl+CMF was conducted with the condition of 6 mg-O_3/L, 25mg-

PAC/L and Flux 4m/d (Figure 4.4 (d)). The operation time was 908 h, and CEB was conducted twice. The turbidity of SE ranged from 4.4 to 22.5 NTU. O_3 consumption and DO_3 were 1.7 to 6.0 mg/L and 0.2 to 1.8 mg/L, respectively. These results, generally low O_3 consumption and high $DO_{3,}$ suggested that 6 mg-O_3/L was relatively high ozone dosage, compared with amount of organic matters in SE. The TMP was well controlled in the range of 25 to 35 kPa during the first 200 h after operation started, but TMP sharply increased over 200 kPa due to a heavy rain. The 1st CEB was conducted to recover TMP after 220 h of operation, and TMP was stable for 313 h after the CEB. The reason why TMP was stable for such a long time can be explained by two reasons. Firstly, the water quality was relatively clear and stable in the operation period, which can be deduced from the results no significant change of O_3 consumption and DO_3. Secondly, high residual ozone could contribute to mitigate fouling by removing foulants as mentioned above. However, TMP increased rapidly from 38 kPa to 63 kPa during 17 h after 514 h of operation. After 2nd CEB, TMP was stably maintained at 40 to 50 kPa for 376 h. In this condition, the CEB interval was estimated as 345 h from the result of this operation.

To sum up, the CEB interval was approximately 24, 48, 90 and 345 h under the condition of 0, 2, 4 and 6 mg/L, respectively, and also the TMP increase rates were 29, 26, 6 and 1 kPa/d, respectively. Moreover, the TMP increase rate decreased largely between the condition of 2 and 4 mg-O_3/L, and it accords with the condition that residual ozone stared to be detected. As mentioned above, the detection of residual ozone means that the ozone started to remain after they degraded most of organic matters in SE, and as a result it was contributed to mitigate ceramic membrane fouling. It was demonstrated that membrane fouling could be alleviated effectively when ozone dosage set over a level that residual ozone is detected.

In addition, the effect of pre-ozonation on reversible and irreversible fouling was investigated using the resistance in series model. Figure 4.5 shows (a) total membrane resistance and (b) the ratio of resistance after initial 24 h of operation.

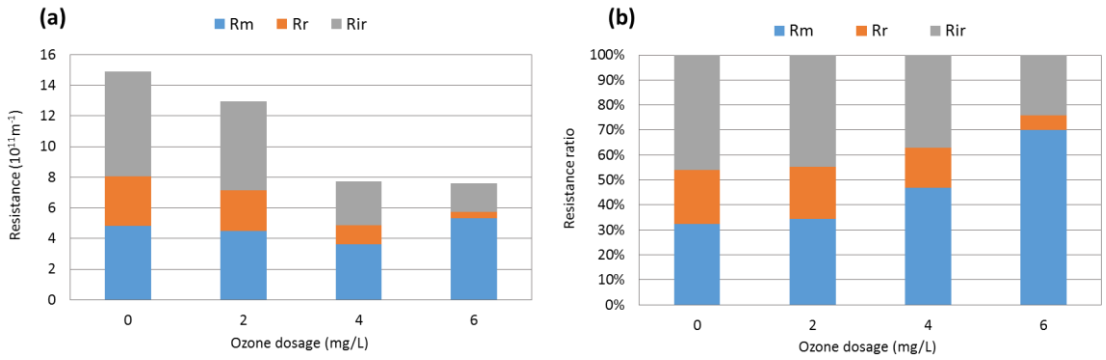


Figure 4.4 Total membrane resistance (a) and the ratio of resistance (b)after 24 h of operation (Rm : Intrinsic membrane resistance, Rr : Reversible fouling resistance, Rir : Irreversible fouling resistance)

R_t was 14.8 x $10^{11}m^{-1}$ under 0 mg-O_3/L (4.8 x $10^{11}m^{-1}$, 3.2 x $10^{11}m^{-1}$ and 6.8 x $10^{11}m^{-1}$ for R_m, R_r and R_{ir}, respectively). However, it decreased to 7.6 x $10^{11}m^{-1}$ with 6 mg-O_3/L (5.3 x $10^{11}m^{-1}$, 0.5 x $10^{11}m^{-1}$ and 1.8 x $10^{11}m^{-1}$ for R_m, R_r and R_{ir}, respectively). Especially, the remarkable reduction was observed between 2 and 4 mg-O_3/L. It coincided with above results that the TMP increase rate decreased largely between the condition of 2 and 4 mg-O_3/L. Regarding resistance ratio, R_f ratio was 67.5 % at the condition of 0 mg-O_3/L (45.9 % and 21.6 % for R_r and R_{ir} ratio, respectively). It decreased to 30 % with 6 mg-O_3/L, and R_r and R_{ir} ratio were 24.3 % and 5.7 %, respectively. Although a majority of decrease was R_r with pre-ozonation, R_{ir} was also remarkably reduced under the condition of 6 mg-O_3/L. According to a recent report, a decrease of R_{ir} by pre-ozonation was negligible while the significant decreases of R_r were observed (Wei et al., 2016). They got rid of residual ozone using KI solution prior to membrane filtration. Thus, it seems that the reduction of R_{ir} was attributed to residual ozone.

As a result, it was found that the CEB interval was prolonged to 345 h (6 mg-O_3/L) from 24 h (0 mg-O_3/L) by incorporating pre-ozonation, indicating that ceramic membrane fouling if effectively mitigated. In basis of this result, energy consumption was calculated in 4.3.3.

4.3.2 Performance of O_3&CMF process for treating primary effluent

4.3.2.1 Water quality items

Table 4.3 Water quality in PACl+CMF+O_3

Date	PAC dosage (mg/L)	Ozone dosage (mg/L)	Samples	TOC (mg/L)	DOC (mg/L)	UV_{254} (cm^{-1})	Turbidity (NTU)
2015/07/28 ~ 2015/07/31	50	10	PE	41.8	34.8	0.351	36.6
			CM permeate	19.5	22.4	0.173	N.D
			Ozonated water	16.5	18.9	0.122	N.D
		30	PE	41.8	34.8	0.351	36.6
			CM permeate	19.5	22.4	0.173	N.D
			Ozonated water	16.9	18.8	0.101	N.D
		50	PE	63.5	43.7	0.211	28.7
			CM permeate	25.7	25.7	0.188	N.D
			Ozonated water	N.A	N.A	N.A	N.D
2015/01/28 ~ 2015/02/01	100	10	PE	70.2	39.9	0.413	60.0
			CM permeate	39.7	40.0	0.210	N.D
			Ozonated water	34.2	34.5	0.155	N.D
		30	PE	70.2	39.9	0.413	60.0
			CM permeate	39.7	40.0	0.210	N.D
			Ozonated water	33.9	34.0	0.131	N.D
		50	PE	63.5	43.7	0.283	31.4
			CM permeate	25.7	25.7	0.190	N.D
			Ozonated water	N.A	N.A	0.104	N.D
2015/05/16 ~ 2015/05/24	150	10	PE	30.6	13.2	0.199	35.2
			CM permeate	21.2	21.1	0.099	N.D
			Ozonated water	17.2	17.2	0.065	N.D
		30	PE	30.6	13.2	0.199	35.2
			CM permeate	21.2	21.1	0.099	N.D
			Ozonated water	17.0	17.2	0.063	N.D
		50	PE	N.A	N.A	0.242	32.2
			CM permeate	N.A	N.A	0.145	N.D
			Ozonated water	N.A	N.A	N.A	N.D

N.D : Not detected
N.A : Not analyzed

Water quality of PE, ceramic membrane permeates and post-ozonated water were summarized in Table 4.4. TOC, DOC and UV_{254} was 30.6 to 70.2 mg/L, 13.2 to 39.9 mg/L and 0.199 to 0.351 cm^{-1} in PE, respectively. 31 to 60% of TOC, 36 to 41% of DOC and 11 to 51 % of UV_{254} was removed by PAC+CM. Also, Turbidity of 31.4 to 60.0 NTU in PE was completely eliminated through PAC+CM. After post-ozonation, the additional 26 to 45% of UV_{254} removal rate was obtained, but TOC and DOC showed no obvious change. Consequently, there is a potential that reclaimed water, produced from PE, would contain TOC, DOC and UV_{254} in range of 16.5 to 34.2 mg/L, 17.2 to 34.5 mg/L and 0.065 to 0.155 cm^{-1}, respectively.

4.3.1.2 Virus removal performance

Figure 4.6 shows MS2 removal rate in (a) PACl+CMF and (b) post-ozonation. The value in Figure 4.6 (a) indicates mean MS2 removal rate, and error bar represents standard deviation. MS2 spike test was triplicated at each PACl dosage.

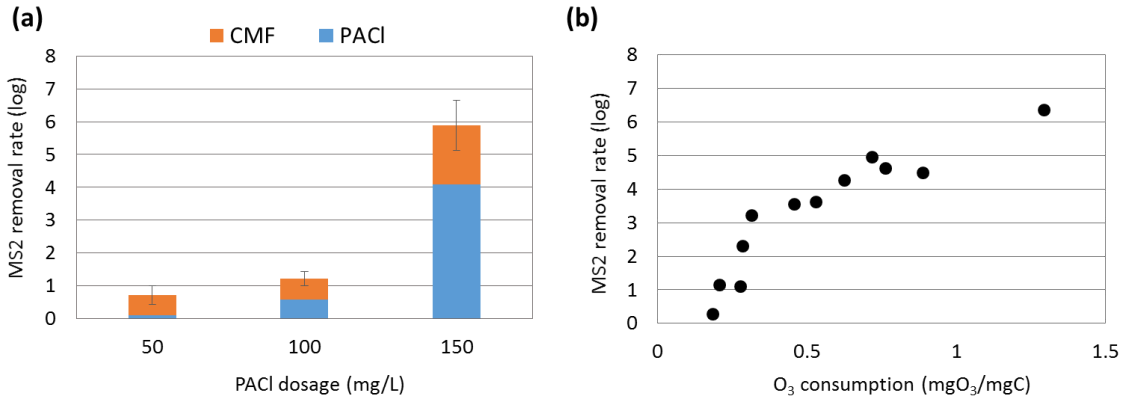


Figure 4.5 MS2 removal rate in (a) PACl+CM and (b) post-ozonation

In PACl+CMF, 6 log of MS2 removal rate was obtained at 150 mg-PACl/L, while the less than 1 log was observed at PACl dosage of 50 and 100 mg/L. Moreover, the removal rate by only coagulation was 0.6 and 4 log under 100 and 150 mg-PACl/L, respectively. From this result, the removal by PACl+CMF was greatly influenced by MS2 coagulation as same with the result in SE. In addition, there was a possibility that MS2 coagulation is variable in accordance with the huge fluctuation in water quality of PE. TOC fluctuated in range of 30 to 70 mg/L as shown in Table 4.4. It indicated that MS2 removal rate is also variable due to the fluctuation of PACl dosage/TOC which showed a high correlation with MS2 removal in 3.3.2.1. Therefore, adequate PACl dosage was necessary to secure the stable virus removal performance in PACl+CMF.

In post-ozonation, meanwhile, MS2 was rarely removed by ozonation (only 0.3 log) until initial 0.2 mgO_3/mgC However, the removal rate increased to 3.6 and 5 log with increasing to 0.5 and 0.7 mgO_3/mgC, repectively. As mentioned in 3.3.1.1, the removal of TOC component such as SS by PACl+CMF could lead to the reduction of ozone dosage. From this result, post-ozonation could be more reliable in removing viruses during PACl+CMF+O_3 for treating PE, compared to PACl+CMF. Thus, virus removal was able to be complemented by post-ozonation even though it was not enough in PACl+CMF.

4.3.2.2 Operational performance

Figure 4.7 shows TMP trend in the continuous operation of PACl+CMF for treating PE. CEB was not conducted in this operation.

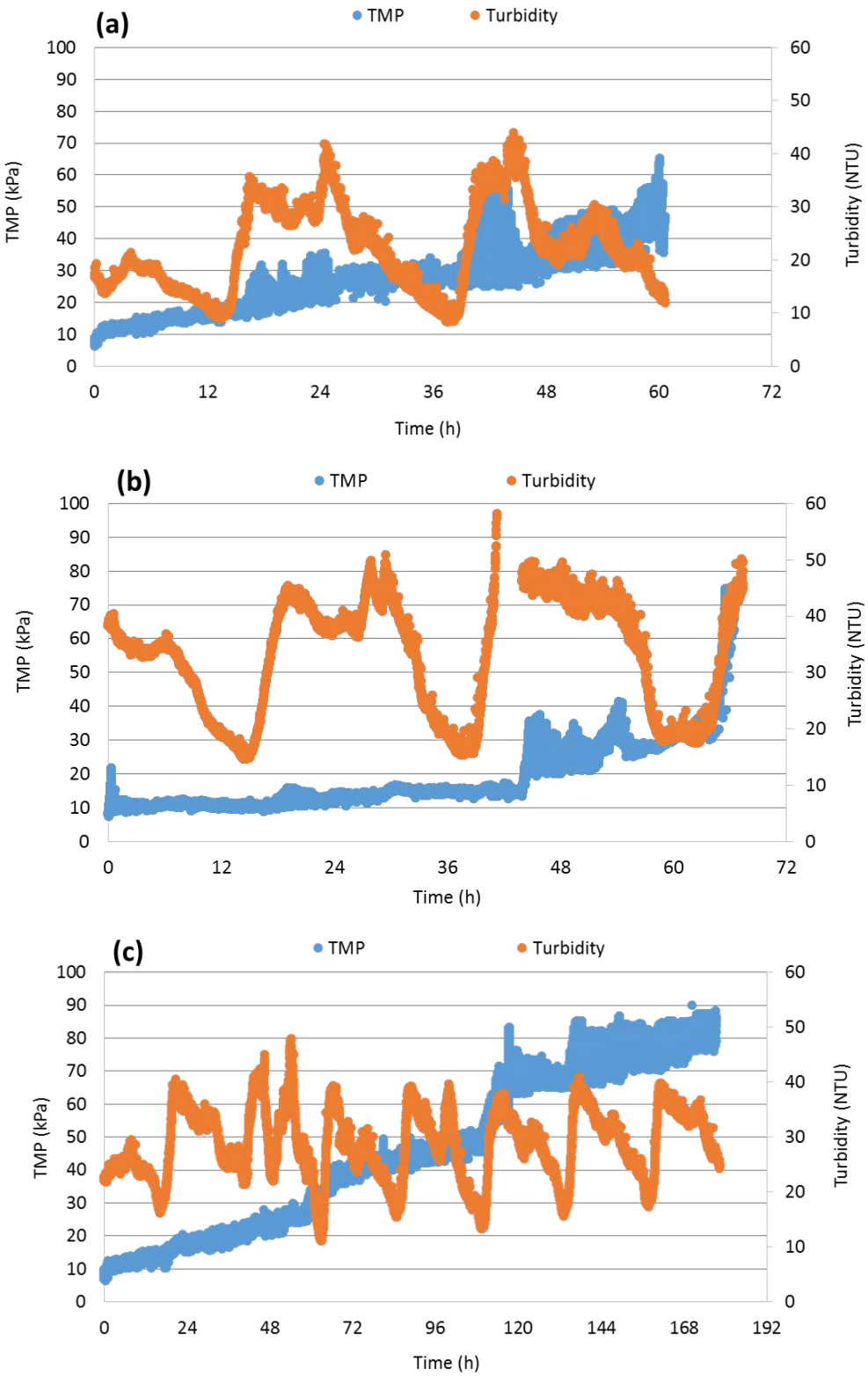


Figure 4.6 Operational performance under PAC dosage of (a) 50 mg/L, (b) 100 mg/L and (c) 150 mg/L

PACl+CMF for treating PE was operated under the condition of 50mg-PAC/L and flux 2m/d (Figure 4.7 (a)). The operation time was about 60 h, and turbidity of PE was in range of 8.5 ~ 47.2 NTU. TMP was gradually increased up to 30 kPa during 48 h of operation. After 60 h, TMP increase rate increased rapidly. A tendency that the increases of TMP in 1 cycle was influenced along with daily turbidity variations was observed.

The operation of PACl+CMF was conducted for 66 h with 100mg-PAC/L and Flux 2m/d (Figure 4.7 (b)). Turbidity of PE was 14.5 to 50.8 NTU. TMP was well controlled in range of 10 ~ 20 kPa for 45 h, even under the turbidity fluctuation. However, turbidity increased over 300 NTU due to heavy rain, and accordingly the operation was temporarily pause, and restarted after turbidity had stabilized. TMP increased from 30 kPa to 75 kPa in 6 h, after 65 h of operation. As mentioned above, it also seems to be due to the formation of homogeneously-distributed pore constriction (Zhu et al., 2012).

PACl+CMF was operated for 177 h with the condition of 150mg-PAC/L and flux 2m/d (Figure 4.7 (c)). Turbidity of PE was 11.2 to 80.6 NTU. TMP gradually increased up to 50 kPa, but its increase rate suddenly increased after 110 h of operation along with the increases of turbidity. After then, TMP gradually increased to 90 kPa without rapid increases. It seems that once evenly developed ceramic membrane pore constriction or clogging was relieved by hydraulic backwashing and turbidity decreases. It was possible to operate stable ceramic membrane filtration for relatively long time under the condition of 150 mg-PAC/L, compared to 50 and 100 mg-PAC/L. As shown in Figure 3.7 (b), zeta potential was close to 0 mV under 150 mg-PAC/L. Charge neutralization of particles by PAC addition caused improvement of coagulation efficiency, thereby mitigating ceramic membrane fouling.

In summary, TMP increase rate was 13.7, 10.7 and 8.6 kPa/d under 50, 100 and 150 mg-PAC/L, respectively. These results indicated that irreversible fouling was alleviated with increasing PAC dosage. Abdessemed and Nezzal. (2002) demonstrated that limit permeate flux increased by 46.6% when 120 mg/L of $FeCl_3$ was added prior to UF for treating PE. However, this result was also obtained through short-term operation (about 3 h), and therefore the process sustainability had remained unclear. In this study, ceramic membrane filtration for treatment of PE was operated for a maximum of 180 h. As a result, it was possible to operate stable ceramic membrane filtration under the condition of 150 mg-PAC/L without rapid TMP increases, while TMP was easily influenced by daily turbidity fluctuation under 50 and 100 mg-PAC/L.

In addition, the influence of PAC addition on reversible and irreversible fouling was

investigated using the resistance in series model. Figure 4.8 shows (a) total membrane resistance and (b) the ratio of resistance after initial 48 h of operation.

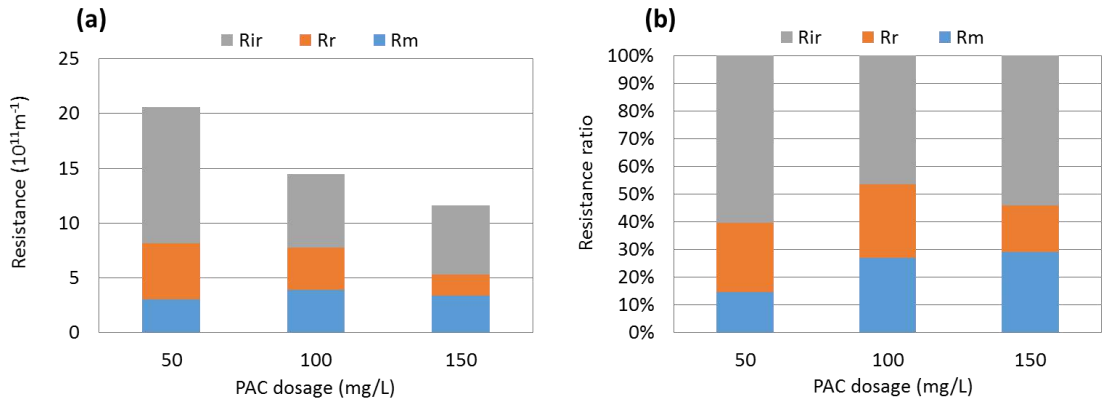


Figure 4.7 Total membrane resistance (a) and the ratio of resistance (b) after 48 h of operation (Rm : Intrinsic membrane resistance, Rr : Reversible fouling resistance, Rir : Irreversible fouling resistance)

R_t decreased from 20.6 x $10^{11}m^{-1}$ to 11.6 x $10^{11}m^{-1}$ with increasing PAC dosage from 50 to 150 mg-PAC/L. R_{ir} decreased to 6.3 x $10^{11}m^{-1}$ from 12.4 x $10^{11}m^{-1}$, and R_r decreased to 1.9 x $10^{11}m^{-1}$ from 5.1 x $10^{11}m^{-1}$. In terms of resistance ratio, R_{ir} ratios were 60.3 %, 46.3% and 54.2 % with 50, 100 and 150 mg-PAC/L, respectively, and also R_r ratios were 24.9 %, 26.7% and 16.7%, respectively. It suggested that the mitigation of R_t between 50 and 100 mg-PAC/L was mainly attributed to the reduction of R_{ir}, whereas the reduction of R_r primarily occurred between 100 and 150 mg-PAC/L. Thus, it seems that the mitigation of irreversible fouling is restrictive when coagulation was only used as pretreatment.

4.3.3 Energy consumption

From the result of continuous operation, energy consumption was calculated in accordance with the method described in 4.2.6. The result of energy consumption calculation was summarized in Table 4.5 (SE) and Table 4.6 (PE).

For SE, energy consumption of PACl+CMF was 0.157 kWh/m^3. By incorporating pre-ozonation, it was possible to reduce energy consumption for CMF from 0.068 to 0.032 kWh/m^3. However, total energy consumption slightly increased to 0.170, 0.192 and 0.216

kWh/m^3 under 2, 4 and 6 mg/L of ozone dosage, respectively.

For PE, 0.157 ~ 0.246 kWh/m^3 of energy consumption was obtained in PACl+CMF. In post-ozonation, a wide range of energy consumption (0.048 ~ 0.238 kWh/m^3) was obtained according to ozone dosage.

On the basis of the result of virus removal performance and energy consumption assessment, the appiliability of reclaimed water produced by O_3&CMF process was investigated based on assumed five expourse scenarios in Table 3.3. Figure 4.9 shows the appiliability of reclaimed water produced by O_3&CMF process for treating SE and PE.

Table 4.4 Energy consumption for treating SE

Treatment process	Operational condition				Energy consumption (kWh/m³)			
	O_3 dosage (mg/L)	PACl dosage (mg/L)	Flux (m/d)	CEB interval (days)	Ozonation	Coagulation	CMF	Total
PACl+CMF	0	25	4	1	0	0.089	0.068	0.157
O_3+PACl+CMF	2			2	0.032	0.089	0.049	0.170
	4			4	0.064	0.089	0.039	0.192
	6			14	0.095	0.089	0.032	0.216

Table 4.5 Energy consumption for treating PE

Treatment process	Operational condition				Energy consumption (kWh/m^3)			
	PACl dosage (mg/L)	O_3 dosage (mg/L)	Flux (m/d)	CEB interval (days)	Coagulation	CMF	Ozonation	Total
PACl+CMF	50	d[a]	2	2	0.113	0.044	d[a]	0.157
	100			4	0.161	0.043		0.204
	150			5	0.209	0.037		0.246
Post-Ozonation	d[a]	3	d[a]	d[a]	d[a]	d[a]	0.048	0.048
		5					0.079	0.079
		10					0.159	0.159
		15					0.238	0.238

[a] depending on the condition of pre-treatment or post-treatment

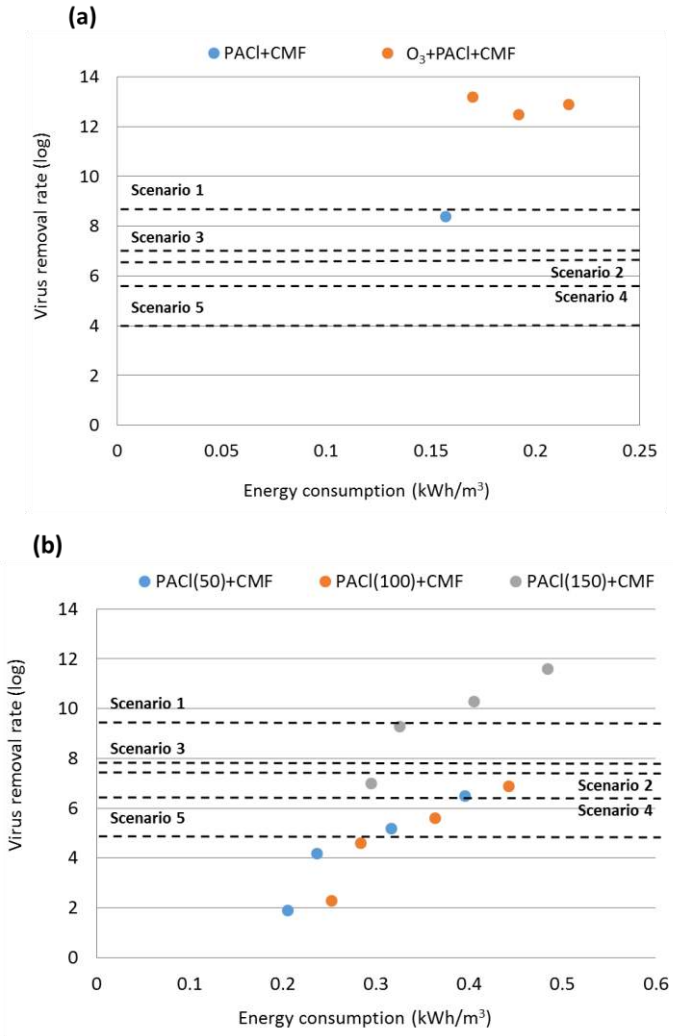


Figure 4.8 The applibility of reclaimed water produced by O_3&CMF process for treating (a) SE and (b) PE

In O_3+PACl+CMF for treating SE, it was difficult to achieve occasionally the target virus removal required in scenario 1 by PACl+CMF. Although energy consumption was slightly increased, higher than 12 log of MS2 removal rate was obtained by incorporating pre-ozonaion. Consequently, it was possible to achieve MS2 removal much higher than that required in all scenarios.

In PACl+CMF+O_3 for treating PE, it was difficult to achieve MS2 removal rate required in scenario 1, 2 and 3 under the relatively low PACl dosage (50 and 100 mg/L). It was found that much higher MS2 removal rate was observed at similar energy consumption under the high PACl dosage (150 mg/L), compared to the condition of low PACl dosage, indicating that it was more efficient from energy aspect. MS2 removal required in all scenarios was satisfied by PACl(150mg/L)+CMF and post-ozonation (> 5 mg/L).

4.4 Conclusions

In this chapter, operational performance of O_3&CMF process for treating SE and PE was evaluated through long-term operation. Virus removal performance was also evaluated using MS2 as the model virus. On the basis of the evaluation, energy consumption was calculated.

Several conclusions can be drawn as follows:

1. In O_3+PACl+CMF for treating SE, > 12 log of MS2 removal rate was observed. In case of operational performance, pre-ozonation successfully mitigate membrane fouling, and as a result the CEB interval was extended from 24 to 345 h with increasing ozone dosage from 0 to 6 mg/L.

2. In PACl+CMF+O_3 for treating PE, 6 log of MS2 removal rate was obtained at 150 mg-PACl/L, and 3.6 and 5 log of MS2 removal rate was obtained by 0.5 and 0.7 mgO_3/mgC of post-ozonation, repectively. In case of operational performance, the CEB interval was estimated as 60 and 180 h under the condition of 50 and 150 mgPACl/L, respectively.

3. In terms of energy consumption, 0.157 ~ 0.216 kWh/m^3 was obtained in O_3+PACl+CMF for treating SE. Although energy consumption was slightly increased, higher MS2 removal rate than that required in all scenarios was achieved by incorporating pre-ozonaion. In case of PACl+CMF+O_3 for treating PE, 0.198 ~ 0.484 kWh/m^3 of energy consumption was obtained. It was found that the relatively high PACl dosage (150 mg/L) was more efficient from energy aspect, compared to the condition of low PACl dosage (50 and 100 mg/L). As a result, MS2 removal required in all scenarios was satisfied by PACl(150mg/L)+CMF and post-ozonation (> 5 mg/L).

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Chapter V

Investigation on the formation of disinfection by-products in ozonation and ceramic membrane filtration combination process and the effectiveness of biological activated carbon treatment addition

5.1 Introduction

It was found that ozonation improves virus removal and membrane operation performance of ozonation and ceramic membrane filtration combination process (O_3&CMF process) from Chapter VI. As mentioned in Chapter II, however, it has been documented that ozonation forms various disinfection by-products (DBPs) known as carcinogens. (Glaze et al., 1987; Richardson et al., 1999; Can&Gurol, 2003; Wert et al., 2006; Haung et al., 2005). Nevertheless, there is insufficient information about formations of DBPs in O_3&CMF process. According to previous researches, moreover, many of these by-products of ozonation, which comprises low molecular weight organic compounds such as organic acids, aldehydes and ketones (Paode et al., 1997; Nawrochi et al., 2003), are easily biodegradable and regarded as assimilable organic carbon (AOC) (Escobar et al., 2001(a); van der Kooij et al., 1989, 1992; Volk and Le Chevallier, 2002; Hammes et al., 2007). AOC is related with bacterial regrowth (van der Kooij et al., 1992; LeChevallier et al., 1992; Escobar et al., 2001(b); 2001(c)). In order to prevent bacterial regrowth in distribution systems, it seems that additional formations of DBPs by chlorine disinfection are inevitable. In water reclamation system, there is a possibility to drink the reclaimed water by mistake depending on their use such as a recreational

impoundment. In addition, reclaimed water has a potential to be used as source of drinking water (Unintended indirect potable reuse) if it was discharged in river. Therefore, it is necessary to investigate formations and controls of DBPs in O_3&CMF process to protect public health.

The aim of this chapter was to investigate not only DBPs formations in O_3&CMF process but formation potentials by chlorine disinfection. Furthermore, the addition of biological activated carbon (BAC) to O_3&CMF process was also studied in order to control DBPs. It is required to control DBPs rigidly depending on the uses of reclaimed water, thereby resulting in the improvement of hygienic safety and the expansion of the uses. BAC following ozonation has proven to be able to significantly remove natural organic matter (NOM), DBPs and their precursors (Asami et al., 1999; Simpson, 2008; Reungoat et al., 2012). Hence, ozonation and BAC process has been widely used as an advanced drinking water treatment technology. However, it is unclear whether *N*-nitrosamines could be removed by ozonation and BAC process, as opposed to the precursors of regulated DBPs (i.e. trihalomethanes or haloacetic acids) which have been well known that they were effectively removed (Karnik et al., 2005; Yan et al., 2010; Chu et al., 2012). According to previous researches, furthermore, microorganisms and particulate matter can penetrate the BAC bed and flow into the effluent (Stringfellow et al., 1993; Han et al., 2013; Zhang et al., 2015). Further studies are therefore required because these microorganisms such as heterotrophic bacteria, and their extracellular polymeric substances (EPS) or soluble microbial by-products (SMP) may accelerate membrane fouling. In this chapter, therefore, the effect on not only the removal of DBPs but also ceramic membrane filtration caused by adding BAC to O_3&CMF process were investigated.

5.2 Materials and Methods

5.2.1 Target DBPs compounds

Four aldehydes (formaldehyde (FAH), acetaldehyde (AAH), butyraldehyde (BAH), propionaldehyde (PAH)), eight *N*-nitrosamines (*N*-nitrosodimethylamine (NDMA), *N*-nitrosomethylethylamine (NMEA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodipropylamine(NDPA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosomorpholine (NMOR), *N*-Nitrosopiperidine (NPIP), *N-Nitroso*di-n- butylamine (NDBA)) and four trihalomethanes (chloroform (TCM), bromodichloromethane, (BDCM), dibromochloromethane (DBCM), bromoform (TBM)) were selected as target compounds

in this study. These target compounds were analyzed by gas chromatography/tandem mass spectrometry (GC-MS/MS). Sample pre-treatments were conducted prior to the GC-MS/MS analysis, and these methods were different depend on their target compounds.

5.2.2 Analytical methods

5.2.2.1 Water quality items

Water quality items were analyzed in accordance with the method described in 3.2.1. Excitation Emission Matrix Fluorescence Spectroscopy (EEM) was measured using spectrofluorometer (Aqualog, Horiba). The excitation and emission wavelength ranges were both 240-800 nm, and EEM fluorescence data were collected for 5nm wavelength of emission at every 3 nm wavelength of excitation. EEM fluorescence data were not normalized to Raman or quinine sulfate units because the relative changes of fluorescence were only examined. EEM spectra was divided into 5 regions according to Chen et al. (2003). Figure 5.1 shows EEM peak used in this study.

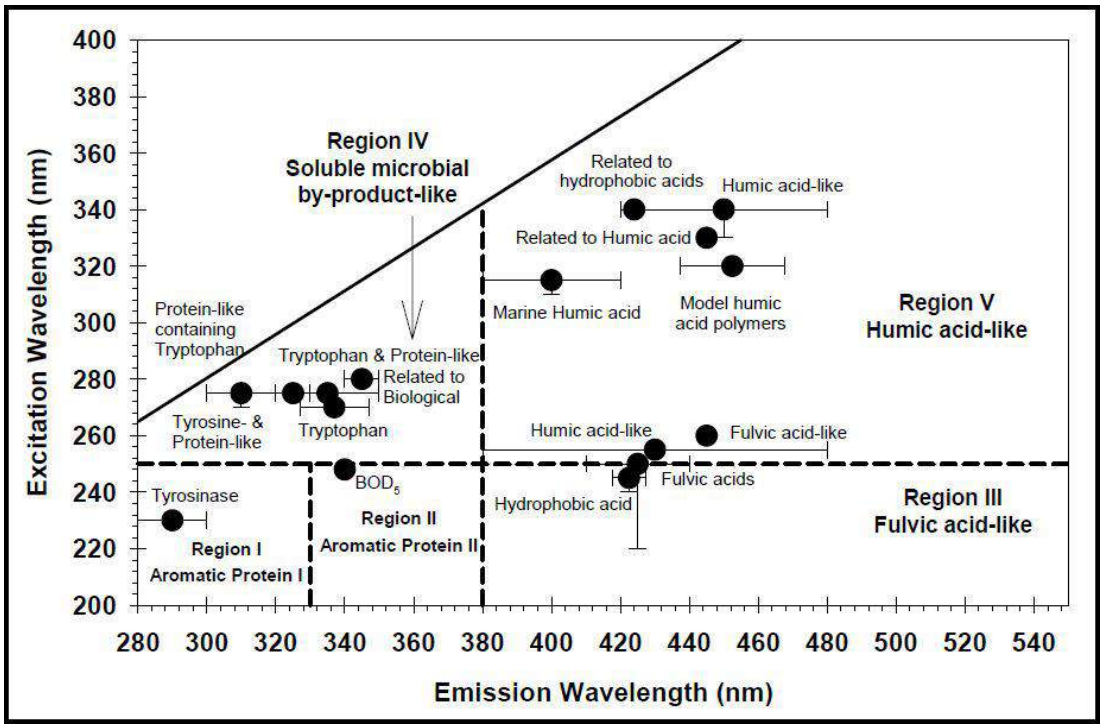


Figure 5,1 EEM peak (Chen et al. (2003))

5.2.2.2 Aldehydes

Aldehydes (ADHs) were analyzed in accordance with a headspace GC-MS/MS method (Sugaya et al., 2001). Figure 5.2 describes the analytical procedure for ADH.

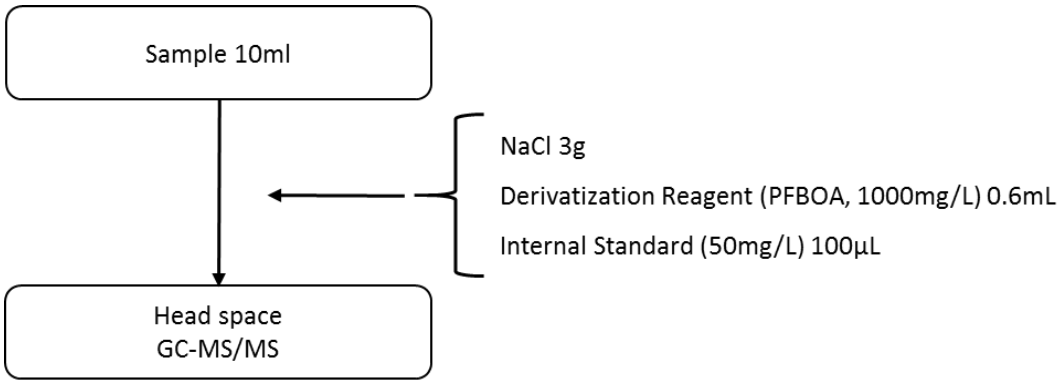


Figure 5.2 Analytical procedure for ADH (Sugaya et al., 2001)

Samples were first filtered by GF/B membrane (pore size 1 μm, Whatman). In case of ceramic membrane filtrate samples were analyzed without GF/B filtration. After filtration, 0.6 mL of o-(2,3,4,5,6- pentafluorobenzyl) hydroxylamine (PFBOA）(1000 mg/L) was added to 10ml of filtered samples as a derivatization reagent. Sodium chloride of 3 g and internal standard solutions of 100 μL (EPA 524.2 Fortification Solution, Supelco) (50 mg/L) were also added. Quantitative analysis of ADHs were conducted using Varian 450 series GC coupled with Varian 300 series MS/MS.

The analytical parameters of GC-MS/MS were shown in Table 5.1.

Table 5.1 Analytical parameters of GC-MS/MS

		Aldehydes	Trihalomethanes	Nitrosamines
GC (Varian 450-GC)	Column	VS-624ms (Varian: 60 m × 0.25 mm × 1.4 μm)	VS-624ms (Varian: 60 m × 0.25 mm × 1.4 μm)	VF-17ms (Varian: 30 m × 0.25 mm × 0.25 μm)
	Oven temperature	60 °C (2 min)– 20 °C/min – 280 °C (7 min)	40 °C (2min)– 30 °C/min– 200 °C (6.5 min)	40 °C (1 min)–5 °C/min– 80 °C–20 °C/min– 280 °C (1 min)
	Flow rate	He (1 ml/min)	He (1 ml/min)	He (1 ml/min)
	Injector temperature	280 °C	200 °C	250 °C
MS (Varian 300-MS)	Detection method	SIM	SIM	MRM
	Ionization mode	NCI (CH4)	EI	PCI (CH4)
	Source temperature	220 °C	220 °C	220 °C
	Transfer line temperature	290 °C	290 °C	290 °C
	Ionization voltage	70 eV	70 eV	70 eV
Injection (Combi PAL)	Injection method	Headspace	Headspace	Liquid injection
	Needele temperature	100°C	100°C	–
	Incubation temperature	60 °C	60 °C	–
	Incubation time	30 min	30 min	–
	Injection volume	500 μl	500 μl	2 μl
	Split mode	20:1	20:1	splitless

5.2.2.3 *N*-nitrosamines

An analytical method, using solid phase extraction (SPE) and GC-MS/MS, previously developed for the determination of *N*-nitrosamines (NAs) in wastewater was employed (Yoon et al, 2012; Takeuchi, 2014). Three deuterated NAs (Cambridge Isotope Laboratories, Inc.) were used as alternative surrogate to quantify eight NAs; Figure 5.3 describes the analytical procedure for NAs.

Samples were first filtered by GF/B membrane and the filtrates were analyzed. In case of ceramic membrane filtrate samples were analyzed without GF/B filtration. Surrogate stock solution was spiked into the samples to obtain 200 ng/L surrogates prior to the SPE procedure. SPE was conducted using Sep-pak NH-2 and AC-2 cartridges (Waters) cleaned with each 5mL of dichloromethane, methanol and Milli-Q water. Samples were then extracted to the SPE cartridges at a flow rate of 10mL/min. After the extraction, the cartridges were rinsed with 30 mL Milli-Q water and dried for approximately 2h. Elution of the analytes from AC-2 cartridges was conducted using 2 mL dichloromethane. The eluents were concentrated under the nitrogen gas stream until just before they dry up. After adding 100 µg dichloromethane and 25 µg toluene-d_8 (CDN Isotope) to the eluent, quantitative analysis of NAs were conducted.

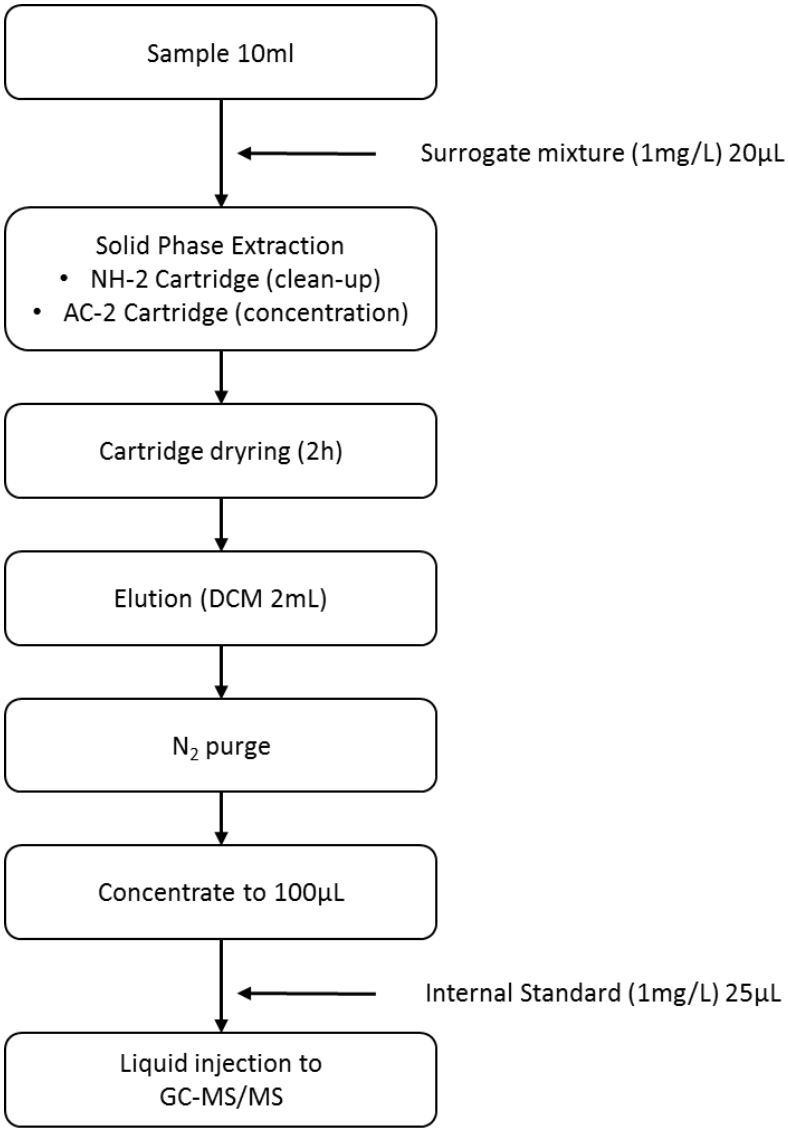


Figure 5.3 Analytical procedure for NAs (Yoon et al, 2012; Takeuchi, 2014)

Table 5.2 Surrogates for each NAs (Takeuchi., 2014)

Analytes	Corresponding surrogate
N-nitrosodimethylamine (NDMA)	NDMA-d_6
N-nitroso-n-methylethylamine(NMEA)	NPYR-d_8 (alternative)
N-nitrosodiethylamine (NDEA)	NPYR-d_8 (alternative)
N-nitrosodi-n-propylamine (NDPA)	NDPA-d_{14}
N-nitrosopyrrolidine (NPYR)	NPYR-d_8
N-nitrosomorpholine (NMOR)	NPYR-d_8 (alternative)
N-nitrosopiperidine (NPIP)	NPYR-d_8 (alternative)
N-nitrosodi-n-butylamine (NDBA)	NDPA-d_{14} (alternative)

5.2.2.4 Trihalomethanes

Trihalomethanes (THMs) were analyzed in accordance with Head space GC-MS/MS method (Japan Ministry of the Environment, 2000; Japan Water Works Association [JWWA], 2001). Figure 5.4 describes the analytical procedure for THMs. The sample pre-treatment procedure for THMs was similar with that for ADHs, except addition of PFBOA. In brief, both 3 g of sodium chloride and 100 μL of internal standard solutions (EPA 524.2 Fortification Solution, Supelco) (5 mg/L) were added to 10ml of filtered sample by GF/B filter. Quantitative analysis of THMs were then conducted using GC-MS/MS.

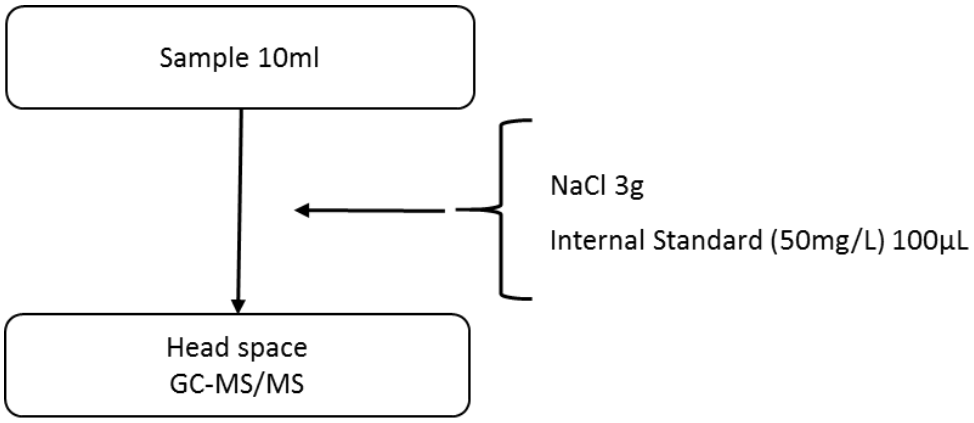


Figure 5.4 Analytical procedure for THMs
(Ministry of the Environment, 2000; JWWA, 2001)

5.2.2.5 General bacteria and Heterotrophic bacteria

In this chapter, both general and heterotrophic bacteria were analyzed in order to investigate the amount of bacteria leaked from BAC treatment. General bacteria and heterotrophic bacteria were analyzed in accordance with standard methods for the examination of water (JWWA, 2011).

5.2.3 Experimental methods for formation potentials

Precursors of target DBPs were evaluated as formation potentials (FP). FP of NAs and THMs, except ADHs, was investigated in this study because chlorine disinfection principally formed halogenated DBPs. FP test followed the procedure described as NAs precursor test by Mitch et al. (Mitch et al., 2003) with minor modification (Yoon et al.,

2012; Takeuchi, 2014). Figure 5.5 describes a schematic diagram of FP test. Monochloramine was prepared freshly before each experiment because of its ability to autodecompose at high concentrations. The free chlorine concentration in the hypochlorite stock solution was determined prior to the preparation of the sodium chloramine solution. Based on the free chlorine concentration in the sodium hypochlorite solution, the volume of sodium hypochlorite stock solution to be added was calculated to obtain a molar ratio of ammonia to free chlorine of 1.2:1 in the final 20mM monochloramine stock solution (around 1400 mg-Cl_2/L). The respective volume of hypochlorite stock solution was added drop wise to the ammonium chloride solution. Monochloramine stock solution (100 mL) was added to unfiltered samples (900 mL) with pH adjusted to 7.0 with 10 mM phosphate buffer. The samples were stirred for 10 days at room temperature with a shaker (TAITEC NR-80) under 100 roll/min. After shaking for 10days, samples were analyzed by the same method shown in 5.2.2. FP of each DBPs was calculated with Eq. 5.1.

$$\mathrm{FP} = C_{10} - C_0 \qquad \text{(Eq. 5.1)}$$

where FP is formation potential of a DBP by chloramine disinfection, C_{10} is the concentration of DBP after 10days, and C_0 is an initial concentration of the DBP.

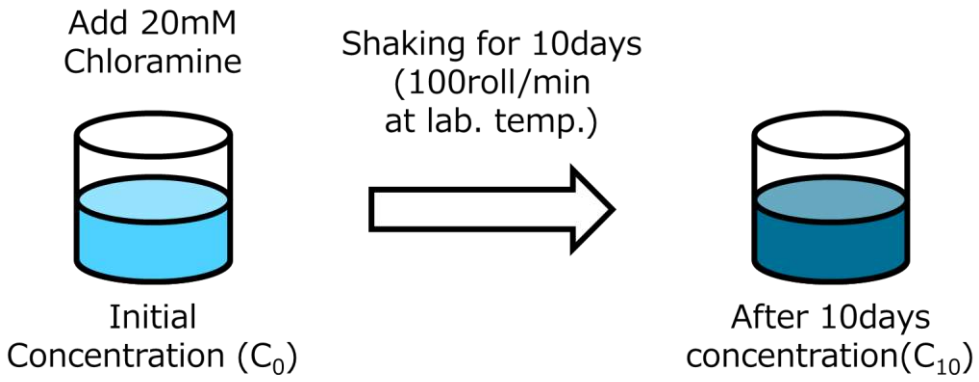


Figure 5.5 Schematic diagram of FP test

5.2.4 Experimental setup of O_3&CMF process without BAC

Experimental setup described in 4.2.2 was used for secondary effluent treatment. Samples for DBPs analysis were collected during continuous operation of O_3&CMF process for treating secondary effluent. In case of primary effluent treatment,

experimental setup described in 4.2.2 and 4.2.3 were used for ceramic membrane filtration and ozonation, respectively.

5.2.5 Experimental setup of O_3&CMF process with BAC

Figure 5.6 shows an experimental setup of O_3&CMF process with BAC.

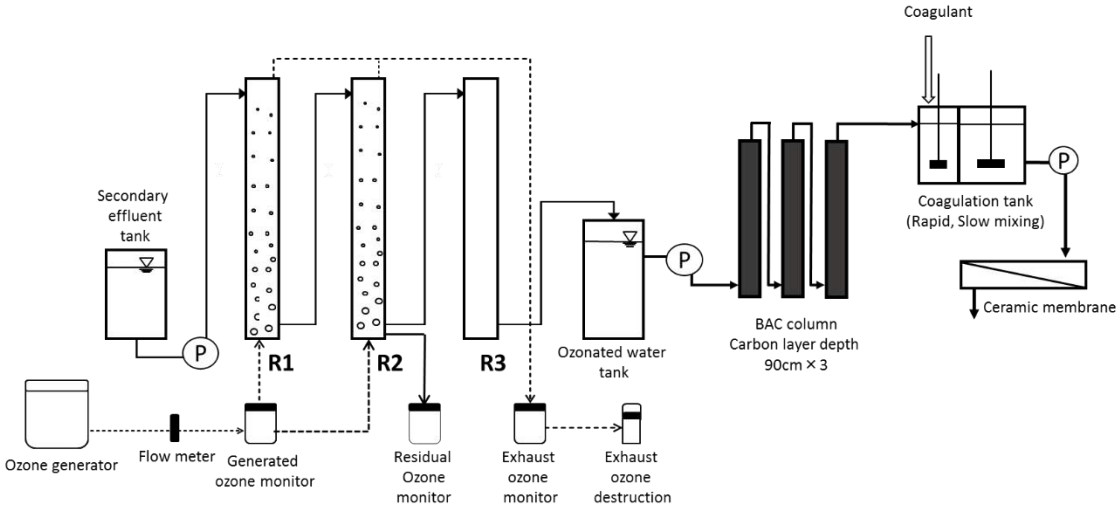


Figure 5.6 Experimental setup of O_3&CMF process with BAC

Table 5.3 The detail of BAC treatment

BAC[a] treatment	
Column Size	ϕ 26mm × 1,000mm
Carbon Bed	900 mm
EBCT[b]	1.5 min × 3
Flow Rate	200 ml/min

[a] Biological activated carbon

[b] Empty bed contact time

Secondary effluent was treated continuously in the order of ozonation, BAC, coagulation and ceramic membrane filtration. Ozonation was conducted using the experimental setup described in 4.2. Ozone dosage was 6mg/L. Ozonated water was flowed to BAC column by peristaltic drive pump (7554-80, Masterflex) at flow rate of 200 mL/min. Three BAC columns, each column was filled with granular activated carbon (034-02125, Wako pure chemical industries) in an acrylic tube, were prepared for BAC treatment. The detail of BAC treatment was described in Table 5.3. Each BAC column

has empty bed contact time (EBCT) of 1.5 min. After BAC treatment, coagulation and membrane filtration was conducted using the experimental setup described in 4.2. Coagulation was conducted on condition of rapid (G=1166 s^{-1}, retention time : 2.5 min) and slow mixing (G=572 s^{-1}, retention time : 5.5 min). Polyaluminium chloride (PACl) (10~11% Al_2O_3, Takasugi pharmaceutical) was used as coagulant, and injected in the rapid mixing tank. PACl dosage was 25mg/L. The ceramic membrane filtration was operated at the constant flux 4 m/d (114 mL/min) in a dead-end mode and continued for 60 min. At the end of each filtration cycle, the ceramic membrane was backwashed at a pressure of 450 kPa with the filtrate for 10s, and was followed by an air blow with compressed air at a pressure of 300kPa.

5.2.6 Ceramic membrane foulants extract and analysis

Ceramic membrane foulants were extracted using NaOH (pH 12) and then HCl (pH 2) after continuous operation of O_3&CMF process. Protein and carbohydrate, known as representative membrane foulants, were analyzed by Lowry method (Lowry et al., 1951) and phenol-sulfuric acid method (Dubois et al., 1956), respectively. These foulants were quantified using bovine serum albumin and glucose as a standard, respectively. EEM was also measured in accordance with the method described in 5.2.2.1.

5.3 Results and discussion

5.3.1 Formation of disinfection by-products in O_3&CMF process for treating secondary effluent

Figure 5.7 ~ Figure 5.9 shows the concentration of DBPs and their FP in O_3&CMF process for treating SE. The experiment was triplicated. The value indicates mean concentrations of DBPs during O_3+PACl+CMF for treating SE. Error bar represents the standard deviation. The legends represent the tested water (SE : secondary effluent, O_3 : ozonated water and O_3+PACl+CMF : ceramic membrane permeate produced from ozonated SE).

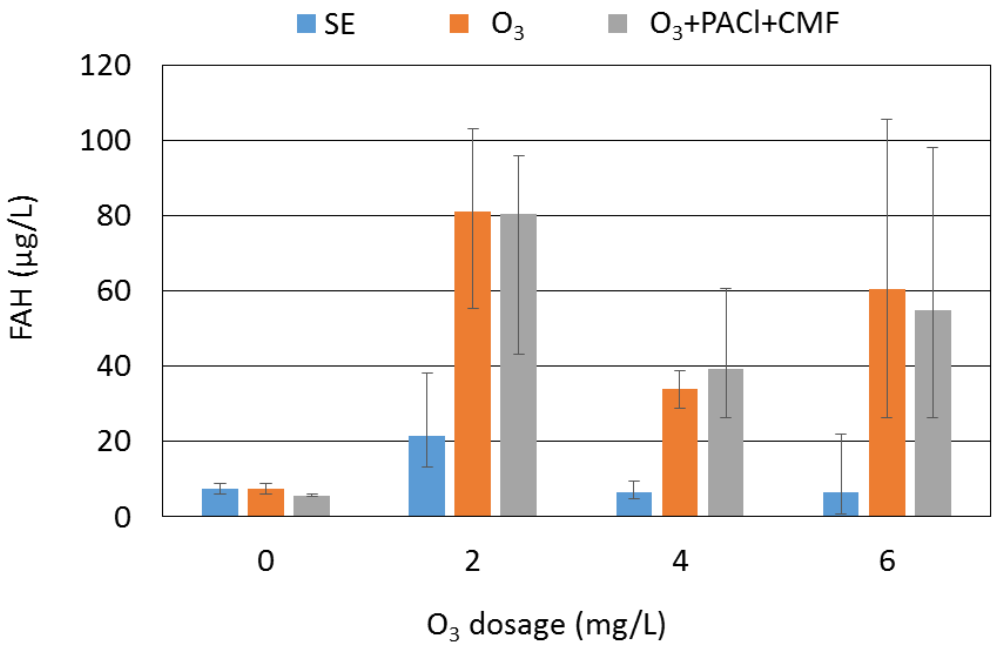
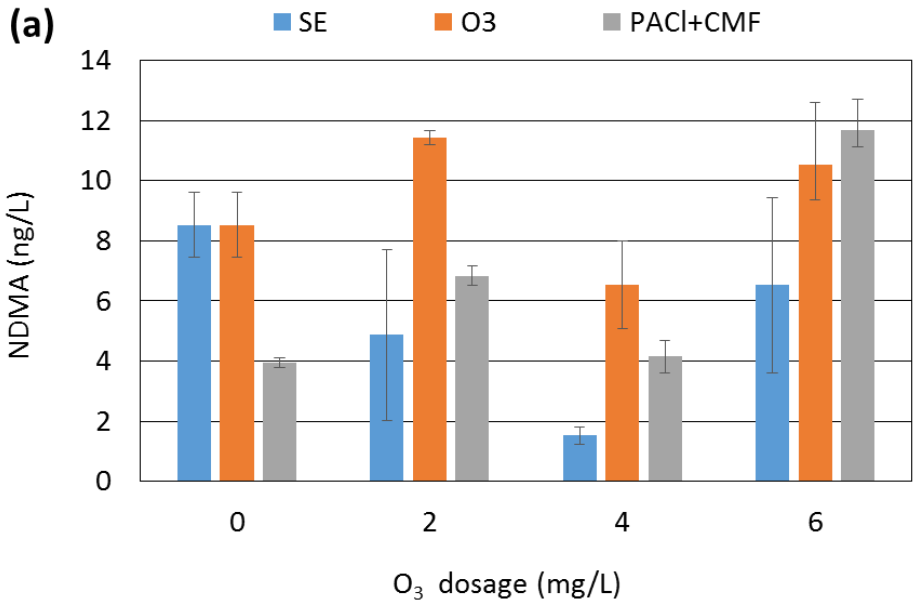


Figure 5.7 FAH concentration in O_3+PACl+CMF for treating SE

FAH shows the largest increase during O_3+PACl+CMF among examined ADHs. AAH, detected below 1 μg/L in SE, was increased slightly at level not exceeding 10 μg/L, and both BAH and PAH were almost not detected during O_3+PACl+CMF (data not shown). The maximum FP of FAH by chloramine disinfection was approximately 15 μg/L (data not shown). This FP was relatively much less than that of NDMA or TCM described in the following Figure 5.8 and 5.9. In addition, it was well known that FAH were formed primarily by ozonation (Glaze et al., 1991; Richardson et al., 1998), hence the FP of FAH was not analyzed in this study.

FAH was present at several tens of μg/L in SE (5.1 to 38.0 μg/L), and then sharply increased after ozonation. As shown in Figure 5.7, the increasing amount of FAH was not necessarily proportional to ozone dosage. The FAH concentration after 4 mg/L of ozonation (34.0 μg/L) was less than that after 2 mg/L (80.9 μg/L) or 6mg/L of ozone dosage (60.6 μg/L). Usually, the concentration of ADHs is proportional to the O_3/DOC ratio, but ADHs may also degrade at higher ozone dosage (Can and Gurol, 2003; Dąbrowska et al., 2005; Huang et al., 2005). Can and Gurol (2003) demonstrated that FAH initially accumulate in water, reach a peak concentration, and then start to degrade by prolonged ozonation. However, FAH start to degrade at ozone dosage higher than 2 mg-O_3/mg-DOC (Can and Gurol, 2003; Huang et al., 2005). This ozone dosage was higher than that of this study (a maximum of 1.5 mg-O_3/mg-DOC in this study). Indeed, the increasing rate was proportional to ozone dosage (277%, 430% and 851% under 2,

4 and 6 mg/L, respectively). It seems that the accumulation of FAH occur rather than degradation during ozonation. From this result, no correlation between ozone dosage and the formation of FAH, arise from the water quality of SE which varies frequently. It has been well documented that DOC characteristics (i.e. UV_{254}/DOC ratio) affect the formation of ADHs (Nawrocki et al., 2003; Karnik et al., 2005; Hammes et al., 2006; Papageorgiou et al., 2014). Thus, the concentration of FAH was determined by not only the ozone dosage condition but also the water quality of SE such as the initial concentration or a precursor of FAH. It means that there is the fluctuation in the formation of FAH even under consistent ozone dosage, hence the monitoring of DBPs is necessary to ensure public health depending on the uses of reclaimed water.



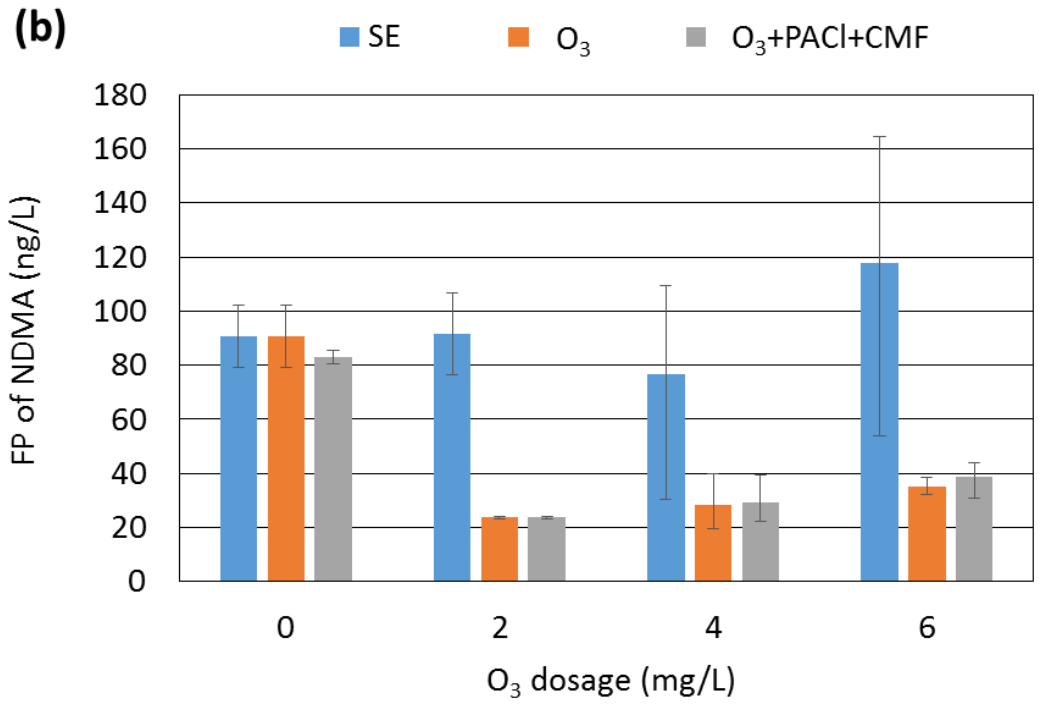


Figure 5.8 NDMA concentration (a) and NDMA FP (b) in O_3+PACl+CMF for treating SE

NDMA was present at concentration of several ng/L in SE, and increased during ozonation up to approximately 12 ng/L (Figure 5.8(a)). NDMA concentration in this study was relatively low compared to the results reported from previous researches. Gerrity et al. (2015) reported that NDMA concentration ranged from 10 to 143 ng/L under ozone dosage ranged from 0.2 to 1.5 O_3/DOC or O_3/TOC. According to Sgroi et al. (2016), however, ozone-induced NDMA formation in SE which has 5.3 mg/L of DOC was 10 ng/L under 0.9 O_3/DOC. This result is quietly similar with our results. They also demonstrated that wastewater treated by extended biological process and nitrogen removal showed low formation of NDMA during ozonation.

The other NAs examined were not detected in SE, and these NAs were not changed after ozonation. NDMA concentration was tend to decrease during PACl+CMF, but slightly increased only under the condition of 6 mg-O_3/L. The residual ozone concentration was 0.8 mg/L to 1.3 mg/L under 6 mg-O_3/L, while it was less than 0.5 mg/L under 2 and 4 mg-O_3/L. NDMA might be formed by the high residual ozone concentration after ozonation. Although ozonation formed approximately 10 ng/L of NDMA, as shown in Figure 5.8 (b), FP of NDMA by chloramine disinfection was dramatically reduced. FP of NDMA ranged from 30.3 to 164.4 ng/L in SE, and decreased to 23.7 ~ 35.1 ng/L after ozonation. 63 ~ 74.3% of NDMA FP in SE was removed by ozonation. It has been

reported that ozonaiton can degrade DBP precursors or alter the chemical properties of them, thereby affecting the formation of DBPs from subsequent chlorination or chloramination (Von Gunten, 2003; Hu et al., 2010; Hua and Reckhow, 2013; Mao et al., 2014; Vera et al., 2015). Although they investigated THMs and haloacetic acid without NDMA, the decrease of NDMA FP indicated in this study seemed to be due to the similar cause. The other NAs examined were not formed even after FP test.

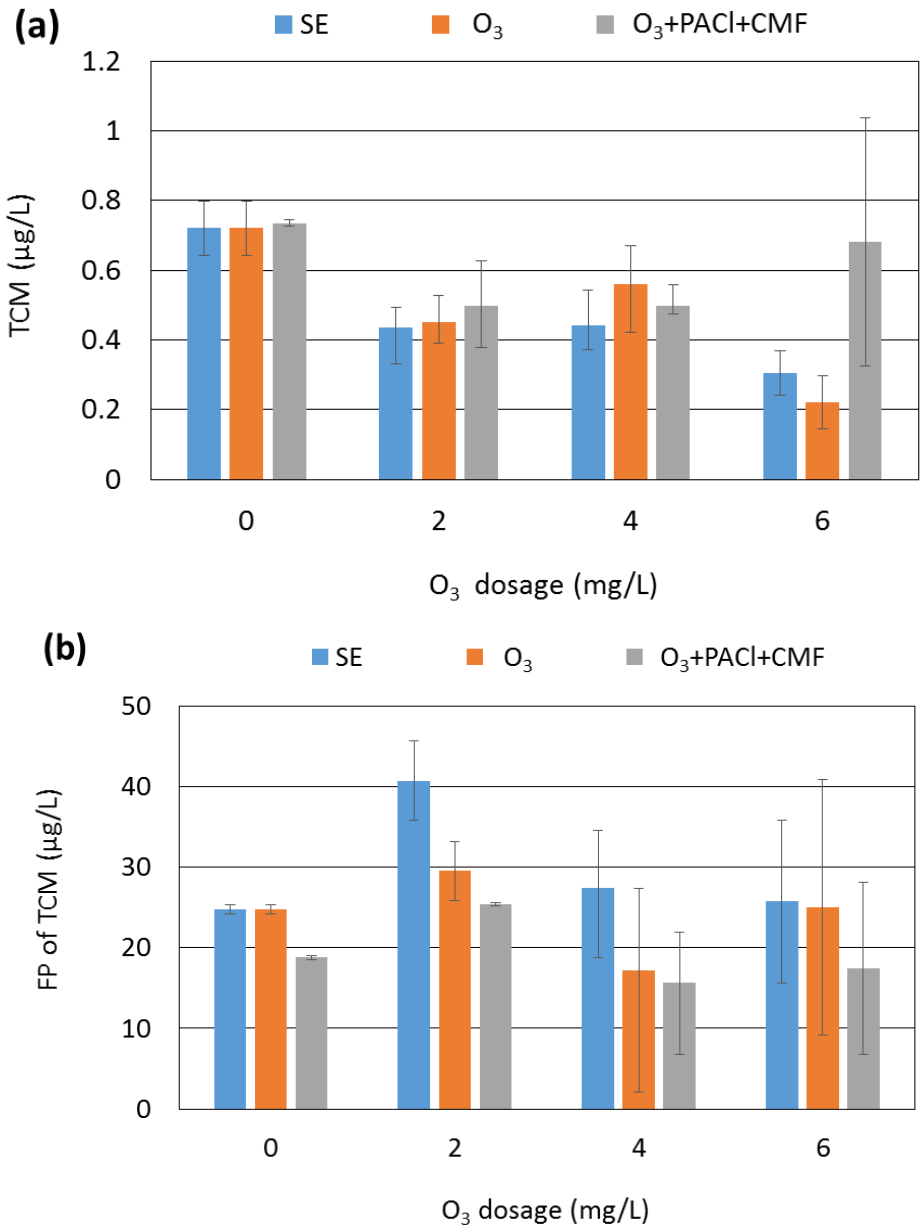


Figure 5.9 TCM concentration (a) and TCM FP (b) in O_3+PACl+CMF for treating SE

In case of THMs, only TCM was detected at concentration less than 1 μg/L in SE, and there was no significant change after ozonation. The other THMs examined, BDCM, DBCM and TBM, was not detected in O_3+PACl+CMF. FP of TCM ranged from 24.7 to 40.7 μg/L. FP of BDCM was approximately 10 μg/L, and FP of DBCM and TBM was less than 10 μg/L. Mao et al. (2014) has reported that THMs FP by chlorination was first increased, reached the maximum at 2 mg/L of ozone dosage, and then decreased with increased ozone dosage. In this study, however, it was found that THMs FP was tend to decrease during ozonation regardless of the ozone dosage. These results were much lower than a regulation value, 80 μg/L as total trihalomethanes, established by U.S EPA (U.S. EPA, 2006). Therefore, FAH and NDMA could be bigger problems than THMs with regard to the use of reclaimed water produced by O_3+PACl+CMF.

In most cases, PACl+CMF could not contribute to remove DBPs because the pore size of ceramic MF membrane was not enough small for DPBs removal. It means that the concentration of DBPs in reclaimed water was almost governed by ozonation.

Although ozonation formed a little amount of NDMA and TCM, as mentioned above, their FP was dramatically reduced. Consequently, it was expected that the formation of halogenated DBPs is able to be inhibited. However, FAH was formed up to a level of concentration which could be a problem on drinking water regulation in Japan (i.e. a regulation value of 80 μg/L) (Japanese Ministry of Health, Labour and Welfare, 2010), and also NDMA concentration was not in compliance with California's potable reuse requirements occasionally (i.e. a notification value of 10 ng/L) (CDPH, 2009). Even though the main use of reclaimed water postulated in this study was not drinking, there is a possibility to drink the reclaimed water depending on their use such as unintended indirect potable reuse. For this reasons, a further study on the removal of DBPs and the effect on ceramic membrane fouling by adding BAC into O_3+PACl+CMF was investigated in 5.3.3 and 5.3.4, respectively, because it is necessary to control DBPs rigorously depending on the use of reclaimed water.

5.3.2 Formation of Disinfection by-products in O_3&CMF process for treating primary effluent

Figure 5.10 ~ 5.12 described the concentration of DBP and their FP in PACl+CMF+O_3 for treating PE. The value indicates the concentration of DBPs and their FP in PACl+CMF+O_3 for treating PE. The legends represent the tested water (PE : primary effluent, PACl+CMF : ceramic membrane permeate produced from PE and

PACl+CMF+O_3 : ozonated ceramic membrane permeate).

FAH ranged from 19.2 ~ 74.6 μg/L in PE, and it decreased (or does not change) after PACl+CMF (Figure 5.10 (a)). It was considered that the decrease by ceramic membrane filtration was attributed to the removal of FAH absorbed in suspended solids (SS). FAH concentrations were 90.1 ~ 222.2 μg/L in ozonated water (i.e. ozonated ceramic membrane permeate), in which it was 2 times higher than that of SE described in 5.3.1. The difference in formation of FAH was caused by not only much higher ozone dosage but also a greater amount of precursors in PE, compared to SE.

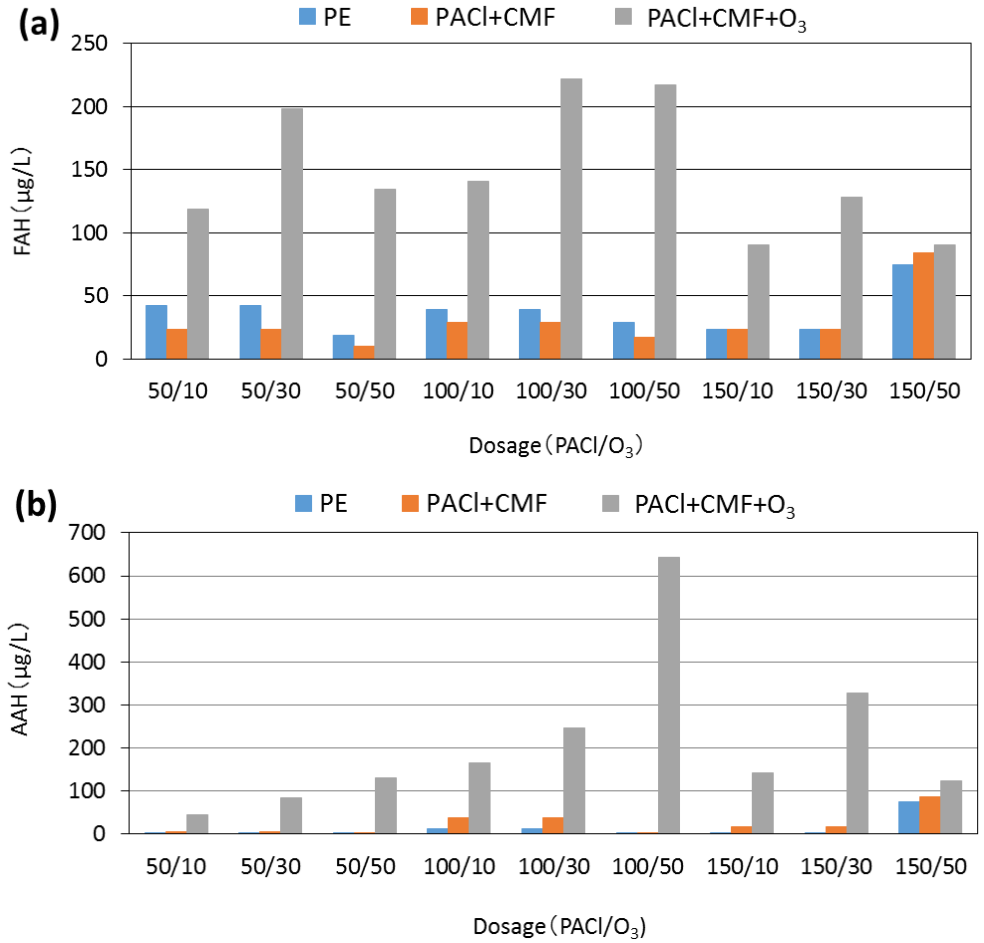


Figure 5.10 Concentrations of (a) FAH and (b) AAH in PACl+CMF+O_3 for treating PE

Meanwhile, AAH was detected at concentrations of 0.54 to 73.6 μg/L in PE (Figure 5.10 (b)). It was also shown that AAH remarkably increased during post-ozonation while there was no change after PACl+CMF. Especially, the maximum concentration of 600 μg/L was

observed under the condition of 100 mg-PAC/50 mg-O_3. In other words, it was expected that reclaimed water produced from PE would contain AAH at the similar level of FAH. BAH and PAH were not detected in PE, but they were formed after post-ozonation. The concentration in ozonated water was 0.88 ~ 19.7 μg/L for BAH and 9.4 ~ 90.9 μg/L for PAH, respectively. According to previous studies, FAH and AAH were primarily observed in ozonated SE (Wert et al., 2007; Tripathi et al., 2011), but it was revealed that BAH and PAH were also detected up to several tens of μg/L in ozonated PE.

Consequently, the maximum ADHs concentration of 971.8 μg/L was observed in reclaimed water produced from PE. As described in Chapter 3, the removal of SS by ceramic membrane filtration result in improving the reaction efficiency of ozone, and as a result the large amount of ADHs was formed during post-ozontion.

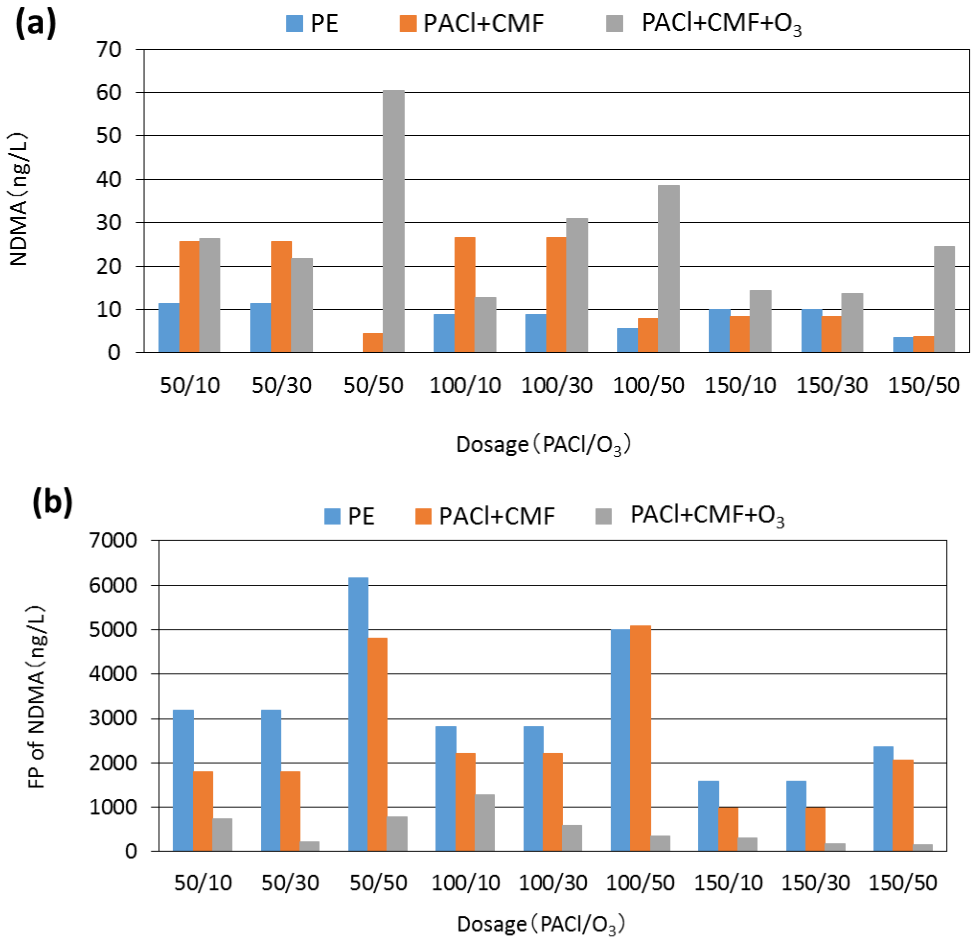


Figure 5.11 NDMA concentration (a) and NDMA FP (b) in PACl+CMF+O_3 for treating PE

In case of NAs, NDMA was detected at concentration level of approximately 10 ng/L in

PE, but the other NAs were not detected even after FP test.

NDMA concentration was similar with that of SE. It seems that NDMA was scarcely degraded during activated sludge treatment. According to Krauss et al. (2009), NAs removal efficiencies in activated sludge treatment were in general above 40% for NMOR and above 60% for the other NAs, but could be lower if concentrations were below 8–15 ng/L in PE. The removal mechanisms of these NAs during activated sludge treatment have been reported to be biodegradation (Sedlak et al., 2005; Fournier et al., 2006), rather than volatilization (Abraham and Al-Hussaini, 2002; Seth et al., 2008) and sorption to SS (Heidler and Halden, 2008; Seth et al., 2008). In terms of cometabolic degradation by monooxygenases, several studies have provided evidence that the transformation rate of a compound depended on the relative concentrations and enzyme affinities of competing substrates (Keener and Arp, 1993; Ely et al., 1997), they thus hypothesize that substrate competition is most likely responsible for the observed threshold concentration for NAs degradation.

NDMA concentration was 12.7 ~ 60.6 ng/L in post-ozonated water, which is lower than that of previous studies. Kosaka et al. (2009) reported that 460 and 1800 ng/L of NDMA was observed after 50 mg/L ozonation of PE filtered with 10 μm polypropylene before ozonation. They explained that the reason why NDMA was detected at high concentration was due to the industrial effluents. In case of PE, in other words, the DBP precursor content has great effect on the formation of NDMA, compared to SE which would has stable water quality through activated sludge treatment. FP of NDMA by chloramine disinfection ranged from 1596.3 to 4991.4 ng/L in PE, but decreased through PACl+CMF and post-ozonation. Especially, ozonation reduced FP remarkably like the results mentioned in 5.3.1, and as a result FP was detected at level of 155 ~ 794.5 ng/L in post-ozonated water. The mechanism by PACl+CMF seemed to be due to the removal of NDMA absorbed in SS, but the results indicated that coagulant dosage has a minor influence on NDMA removal. In case of ozonation, the degradation of NDMA precursors was considered as a major cause, and the removal rate of FP increased with increasing ozone dosage (42 ~69 %, 74 ~ 88% and 83~93% at the ozone dosage of 10 mg/L , 30 mg/L and 50 mg/L, respectively).

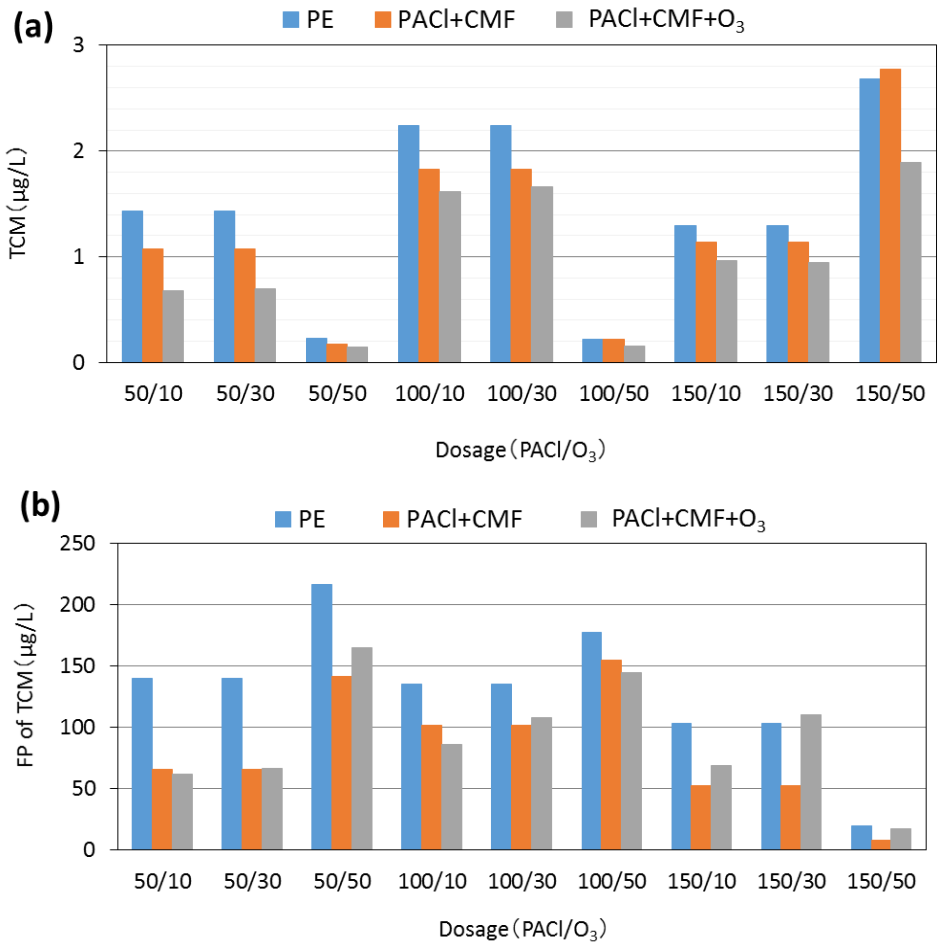


Figure 5.12 TCM concentration (a) and TCM FP (b) in PACl+CMF+O_3 for treating PE

TCM was observed at a maximum concentration of 2.7 μg/L in PE, while the other THMs were not detected. The concentration of TCM slightly decreased after PACl+CMF and ozonation. It was found that ozonation form rarely THMs from the results of both PE and SE, which was similar with the results of previous studies (Richardson et al., 2007; Mao et al., 2014). Meanwhile, FP of TCM was observed ranging from 19.5 to 217 μg/L and from 17 to 144 μg/L in PE and post-ozonated water, respectively. FP was tend to decrease during PACl+CMF, whereas there was no significant change or increased under some conditions after ozonation. It was also found that FP was not proportional to ozone or PAC dosage. Moreover, BDCM, DBCM and TBM, which have the concentrations below than 10 μg/L after FP test in SE, were detected at level of 21.3 ~ 52.5, 19.4 ~ 33.8 and 13 ~ 61 μg/L, respectively (Supplementary material S.5.1). Consequently, reclaimed water has a possibility to contain total THMs at the maximum concentration of 291.3 μg/L.

In conclusion, it was expected that reclaimed water produced from PE by PACl+CMF+O_3 could contain both ADH and THMs at concentrations of several hundreds of μg/L. In addition, even though ozonation could reduce FP of NDMA, approximately 1000 ng/L of NDMA was remained in reclaimed water after chlorination. These DBPs in reclaimed water have much higher concentration values than various guideline or regulation values, established by some countries. For example, the estimated NDMA concentration in this study was about 100 times higher than the notification value of CDPH and the guideline value established in Australian Guidelines for Water Recycling (10 ng/L). There is a risk that high NDMA concentration could cause health problems to the users who was exposed to reclaimed water.

In case of PE, therefore, it is recommended that the utilization of reclaimed water should be restricted to the use which has less possibility to be exposed to users. For this reason, the minimum ozone and chlorination dosage, at levels of the removing odors or colors and preventing the regrowth of microorganism respectively, should be firstly considered, rather than the addition of another treatment to control DBPs formed during PACl+CMF+O_3.

5.3.3 Control of disinfection by-products in O_3&CMF process with BAC treatment

The control of DBPs by O_3+PACl+CMF with BAC was investigated. Figure 5.13 shows the removal of DBPs and FPs in O_3+PACl+CMF with BAC. The value indicates the concentrations of DBPs and their FP.

FAH concentration, 11 μg/L in SE, increased up to 92.5 μg/L after 6mg/L of ozonation, while it decreased gradually by passing through each BAC column, and as a result decreased to 20.5 μg/L after 4.5 min EBCT of BAC (Figure 5.13 (a)). In case of NDMA, the concentration was 18.3 ng/L after ozonation, and then decreased to 10.5 ng/L by BAC (Figure 5.13 (b)). TCM was not detected after ozonation (data not shown). Approximately 77.8% and 42.6% of FAH and NDMA removal rate, respectively, were obtained during BAC treatment even under the condition of short EBCT like 4.5 min. These results demonstrated that FAH was easy to be removed by BAC compared to NDMA, which seemed to be due to the difference in biodegradation between them. It has been well known that FAH is a biodegradable compound (Gerike and Gode, 1990; Eiroa et al., 2004). According to a previous study, FAH is readily biodegradable in aquatic environments whereas photolysis do not occur (板井, 2016). On the other hands, NDMA

is easily degraded by photolysis, and moreover it do not readily sorb to sediments and is slow to biodegrade in soils, sediments, and surface water (Mallik et al., 1981; Kaplan et al., 1985; Yang et al., 2005; Plumlee et al., 2007).

Meanwhile, FP of NDMA, 141 ng/L in SE, decreased to 40 ng/L after ozonation, and then slightly decreased to 27 ng/L during BAC (Figure 5.13 (c)). FP of TCM was no change after ozonation, but it decreased by BAC from 42.2 ng/L to 23.4 ng/L (Figure 5.13 (d)). Ozonation could cleave the unsaturated bonds in aromatic moieties that are found in NOM, which makes the organic molecules smaller, and more biodegradable (Lee et al., 2009; Yan et al., 2010; Chu et al., 2014). Thus, ozonation chemically altered the molecular structures of the precursors, and consequently made the DBPs precursors easier to remove in subsequent BAC.

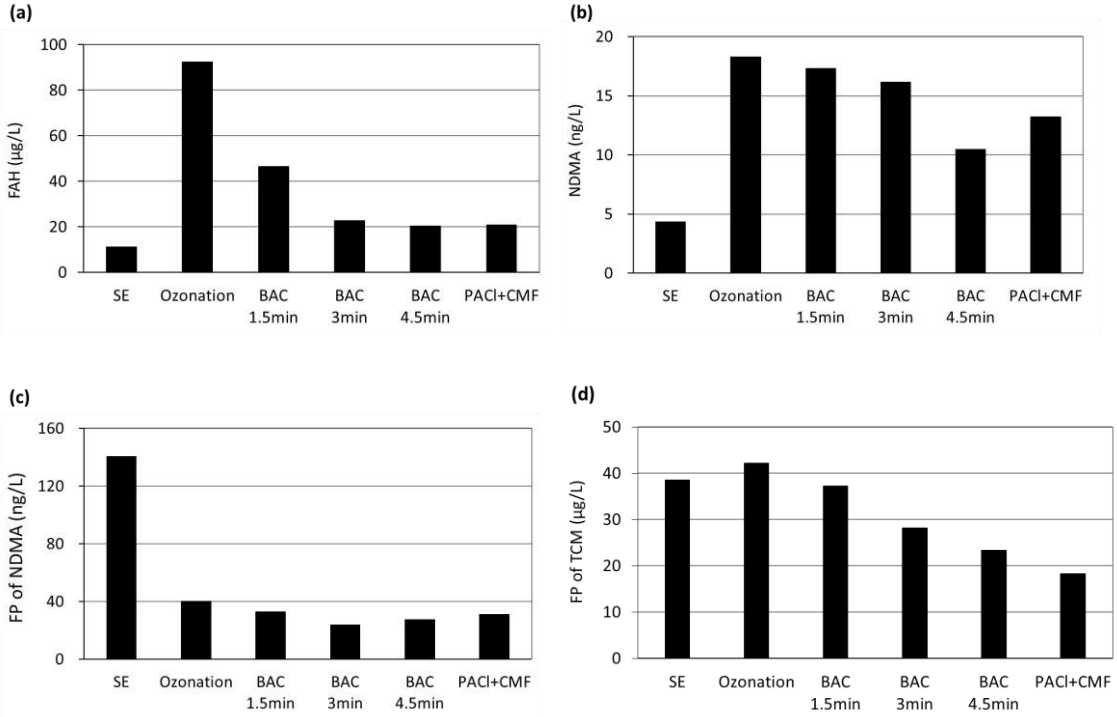


Figure 5.13 The concentrations of (a) FAH, (b) NDMA and FP of (c) NDMA, (d) TCM in O_3+PACl+CMF with BAC

These results indicated that the DBPs concentration tends to decrease with increasing EBCT. Therefore, the removal rate of DBPs was investigated with extended EBCT of BAC. BAC treatment was conducted with decreased 40 mL/min of flow rate (200mL/min before), thereby resulting in the extension of EBCT to 22.5 min. Figure 5.14 described

the DBPs removal under extended EBCT of 22.5 min. The value indicates the concentrations of FAH and NDMA FP.

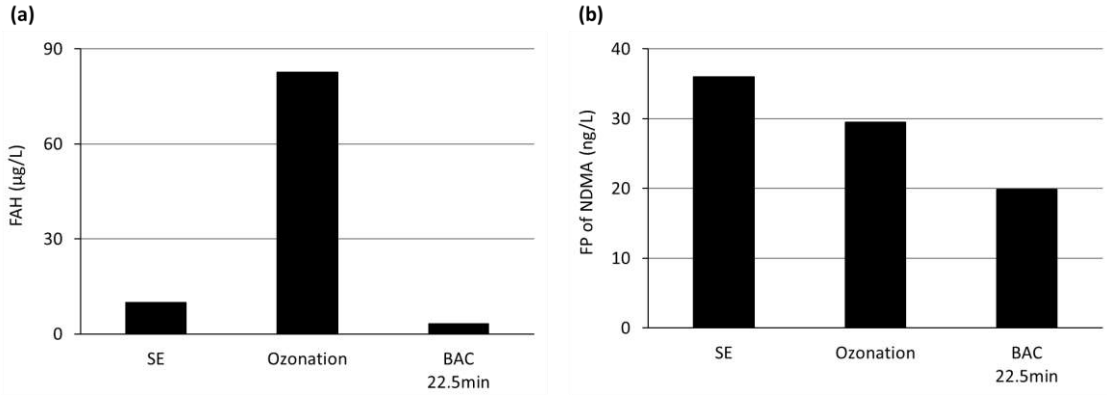


Figure 5.14 The removal of (a) FAH and (b) NDMA FP by BAC under extended EBCT

FAH, 82.7 μg/L after ozonation, decreased to 3.3 μg/L by extended EBCT of BAC. The concentration of FAH after BAC was much lower than that of SE. Moreover, FP of NDMA decreased from 29.5 to 19.9 ng/L after BAC. In case of FAH, moreover, much higher removal rate was obtained under extended EBCT, compared with 4.5 min, whereas NDMA was no change. However, Pramanik et al. (2015a) demonstrated that FP of NDMA, 125 ng/L in SE, was not detected after BAC treatment under 30 min of EBCT. It was considered that a long enough EBCT is needed in order to remove NDMA FP effectively.

Consequently, the extension of EBCT can improve the removal of both DBPs and their FP by BAC. Thus, the investigation of appropriate EBCT condition is required through future studies.

5.3.4 Effect of adding BAC treatment on ceramic membrane fouling

Although BAC treatment has a potential to remove DBPs effectively, microorganisms and particulate matter leaked from the BAC bed could accelerate the fouling of subsequent ceramic membrane filtration. Therefore, the effect of the addition of BAC treatment to O_3+PACl+CMF on ceramic membrane filtration was investigated. The continuous operation of O_3+PACl+CMF with BAC was conducted in accordance with experimental setups and methods described in 5.2.5.

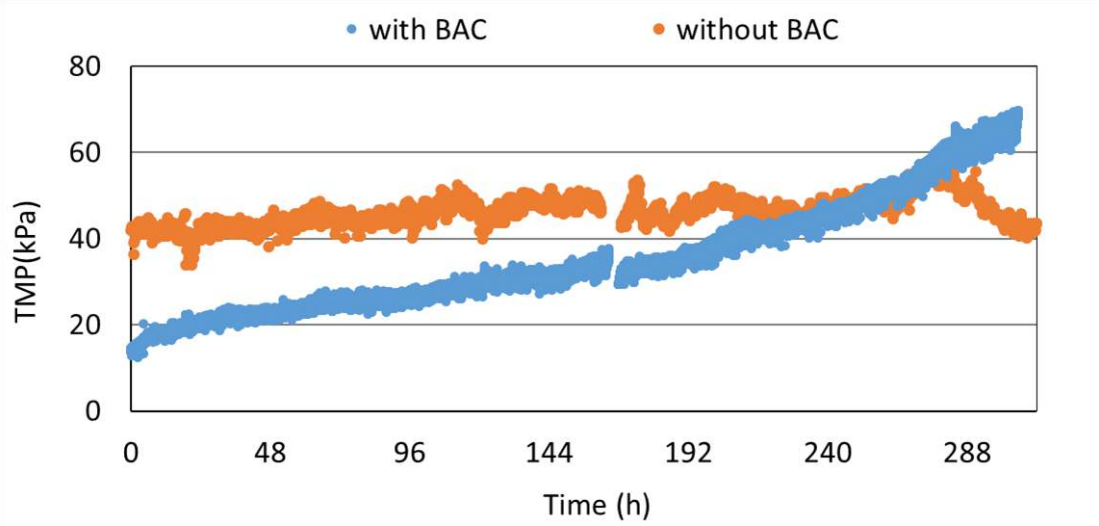


Figure 5.15 The comparison of TMP

Figure 5.15 shows the comparison of TMP between O_3+PACl+CMF with and without BAC (w/ BAC and w/o BAC). TMP of w/o BAC in Figure 5.15 is the some part of results described in Figure 4.4 of Chapter IV (TMP from 531 to 908 h). The experiment of w/o BAC was started about 22 days before the launch of w/ BAC. The ceramic membrane used for the w/o BAC was washed by chemicals (CEB) in accordance with the method described in 4.2.3.1, and then the operation was restarted in parallel with the launch of w/ BAC. Therefore, initial TMP was about 40 kPa, which was higher than that of w/ BAC, but TMP increase rate was quiet small, less than 0.1 kPa/day. While TMP in w/ BAC was below than 20 kPa at the initial stage, it increased up to 70 kPa after 13 days of continuous operation, and the TMP increase rate, 4 kPa/day, was higher than that of w/ BAC. In addition, TMP increase rate became gradually increased as time passed, which is due to similar reasons demonstrated in 4.3.1.2.

These results indicated that membrane fouling was accelerated by addition of BAC treatment, and it can be explained by following two reasons. Firstly, the residual ozone decreased through BAC. As mentioned in chapter 3 and 4, the residual ozone still remained at relatively high concentration in ceramic membrane feed water (coagulated water) under higher ozone dosage in w/o BAC, thereby resulting in the removal of fouling matters or the inhibition of biofouling formation. However, the residual ozone, which ranged from 0.8 to 1.2 mg/L after ozonation, decreased to less than 0.5 mg/L through BAC, which may cause negative effects on membrane fouling. Secondly, a leakage of microorganisms and organic materials can occur and be present in BAC effluent. It has been well documented in previous researches (Stringfellow et al., 1993; Lin et al., 2010; Han et al., 2013; Zhang et al., 2015).

Thus, the amount of microorganisms leaked from BAC was investigated. Figure 5.16 shows the concentration of (a) general bacteria and (b) heterotrophic bacteria in w/ BAC.

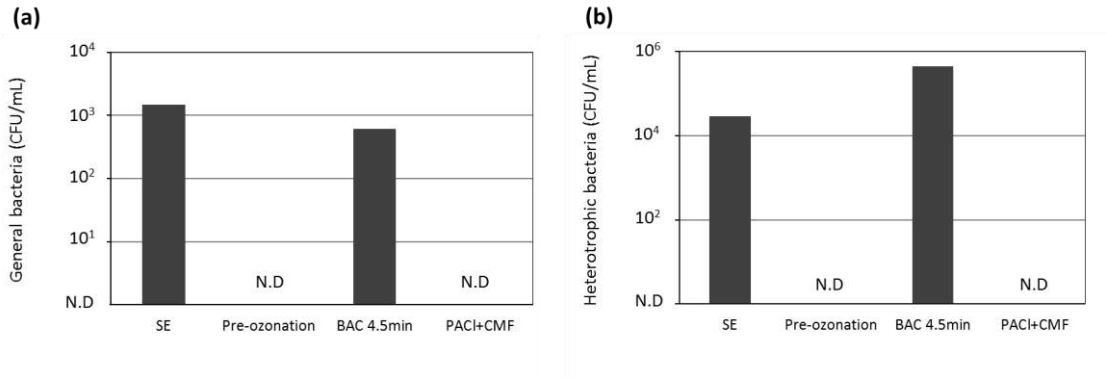


Figure 5.16 A leakage of (a) general bacteria and (b) heterotrophic bacteria from BAC

The concentration of general bacteria and heterotrophic bacteria was about 10^3 and 10^4 CFU/mL in SE. Both general and heterotrophic bacteria was not detected after ozonation, but they was observed at similar levels of SE after BAC. These results indicate that large amount of bacteria released from BAC contributes to acceleration of membrane fouling.

To sum up, the inactivation of bacteria and high residual ozone contributed to inhibit the formation of biofouling in w/ BAC. In w/ BAC, on the other hand, increases the biodegradability by ozonation and the leakage of bacteria from BAC could lead to increase the risk of biofouling. It seems that these differences between ozonation and ozonation followed by BAC as pretreatment of ceramic membrane filtration had influence on TMP increase rate.

Meanwhile, BAC has the potential for effective foulant removal because it could remove organic matters in SE by both adsorption and biodegradation (Pramanik et al., 2015a; 2015b). Even though some bacteria were released from BAC, the removal of organic matters by adsorption and biodegradation was also expected at the same time. For this reason, the behavior of dissolved organic matters in w/ BAC was investigated through EEM spectra. Figure 5.17 describes the change of EEM spectra in w/ BAC.

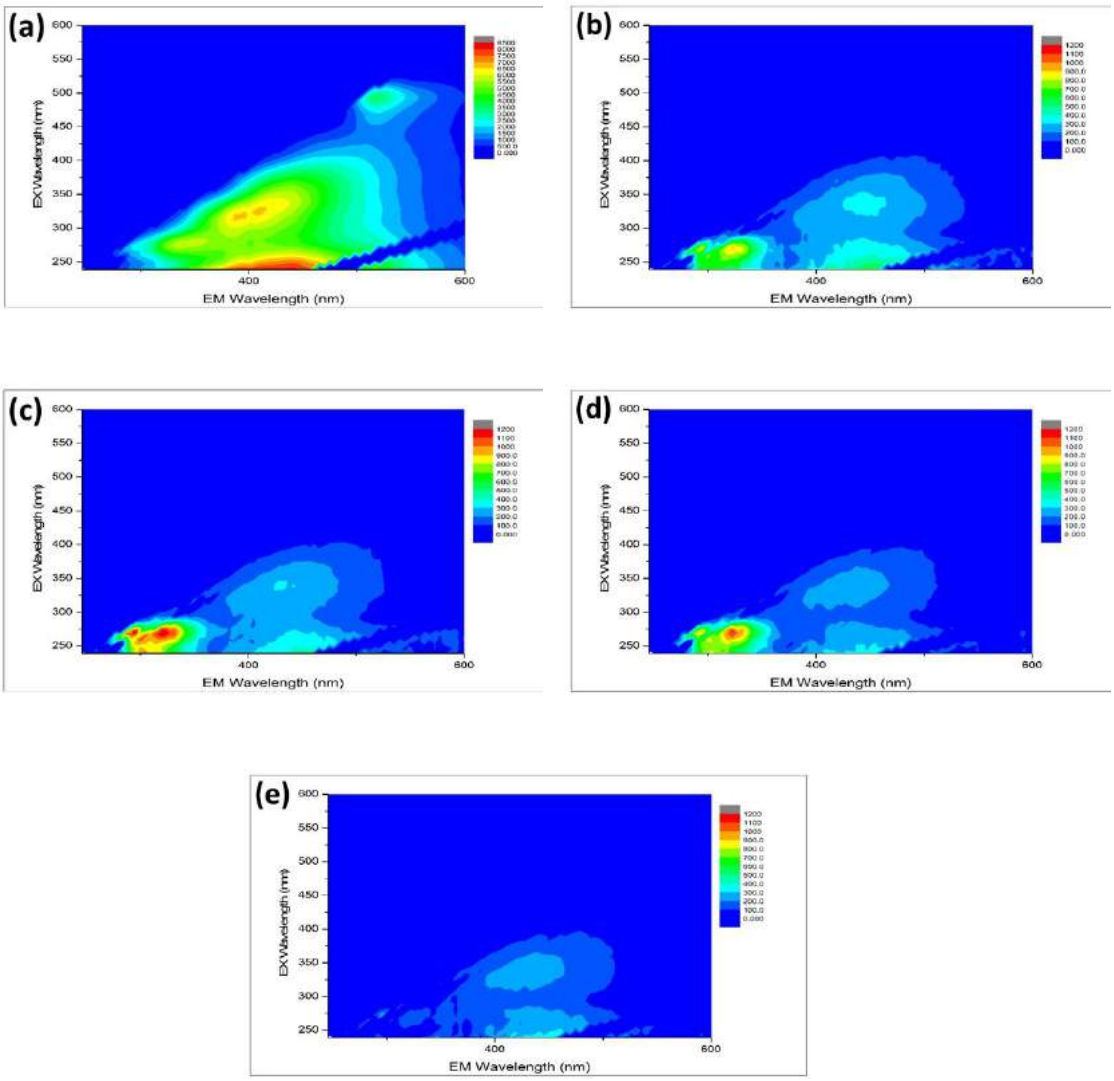


Figure 5.17 EEM spectra of (a) SE, (b) Pre-ozonated water, (c) BAC effluent, (d) Coagulated water and (e) CM permeate

Large reductions of the fluorescence response in all regions was observed after ozonation. While the peak intensity of regions III (Fulvic acid-like materials) and V (Humic acid-like material) decreased, regions IV (Soluble microbial by-product-like material) slightly increased after BAC. Ceramic membrane filtration significantly reduced the fluorescence response of regions I, II (Aromatic protein) and IV, whereas regions III and V showed no obvious change between feed and permeate, indicating that aromatic proteins and SMP were major foulants.

BAC could remove humic and fuvic acid-like materials by adsorption and biodegradation, but these material relatively have not a large contribution to membrane

fouling than SMP like materials. On the contrary, SMP-like materials, which was considered as major foulants, were released from BAC, and flow into BAC effluent. However, these results does not provide information on the amount of dissolved organic matters leaked from BAC, thus further studies on a quantitative analysis was required.

According to Nguyen and Roddick. (2010), BAC following ozonation was able to improve the membrane flux, but it was mainly caused by lowering the total suspended solid level of the ozonated water. Also, the hydraulically irreversible fouling was reduced after ozonation while BAC did not contribute to a further decrease in this type of fouling. Furthermore, they has been pointed out that the combined ozonation and BAC as pretreatment may accelerate biofouling in subsequent membrane filtration because ozonation generated easily biodegradable organic components, which were not removed completely by BAC. However, these presumptions were not verified through long term operation of membrane filtration. Our results coincided with previous results, and especially could provide information on the effect of BAC on TMP through continuous operation.

In addition to above results, EEM spectra of foulants extracted from ceramic membrane after continuous operation using both citric acid and NaOH was analyzed in order to compare the irreversible foulants in w/ BAC and without BAC. Figure 5.18 shows EEM spectra of extracted foulants from ceramic membranes, which were used for (a) w/ BAC and (b) w/o BAC.

The peak intensity scale of Figure 5.18 (a) was discord with that of (b), because there is difference in the scale of ceramic membrane. Lab (effective area 0.042 m^3) and pilot scale (effective area 0.42 m^3) of ceramic membrane was used for w/ BAC (Figure 5.18 (a)) and w/o BAC (Figure 5.18 (b)). Accordingly, the total permeate volume in (b) was 10 times greater than in (a).

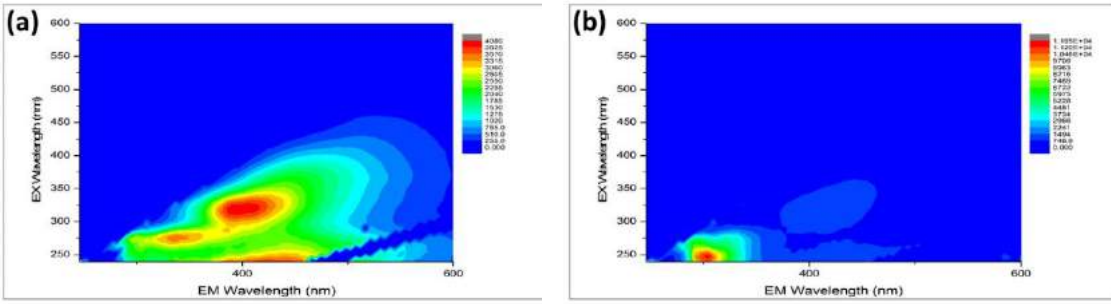


Figure 5.18 EEM spectra of extracted foulants from ceramic membrane used for (a) w/ BAC and (b) w/o BAC

The peak in regions III, IV and V was observed in Figure 5.18 (a), which indicates that extracted foulants from ceramic membrane used for w/ BAC were composed with mainly SMP, humic and fuvic acid-like materials. On the other hand, regions I and II showed the highest peak intensity in Figure 5.18 (b), though the fluorescence response of regions III and V was weakly detected. Aromatic proteins seemed to be major foulants of ceramic membrane used for w/ BAC. The peak in regions IV was a prominent difference between (a) and (b), which demonstrated that the contribution to irreversible fouling by SMP like-materials increased, in case of the addition of BAC as pretreatment. These results that the fluorescence response of SMP like-materials was detected in extracted foulants from ceramic membrane used for w/ BAC corresponded to above results such as the leakage of microorganisms and the increases of the peak intensity in regions IV after BAC.

Furthermore, protein and carbohydrate content in the extracted foulants was analyzed by Lowry method and phenol phenol-sulfuric acid method, respectively, for quantitative comparisons. Both protein and carbohydrate has been well known as major foulants (Jarusutthirak et al., 2002; Laabs et al., 2006; Yamamura et al., 2007; Fan et al., 2008).

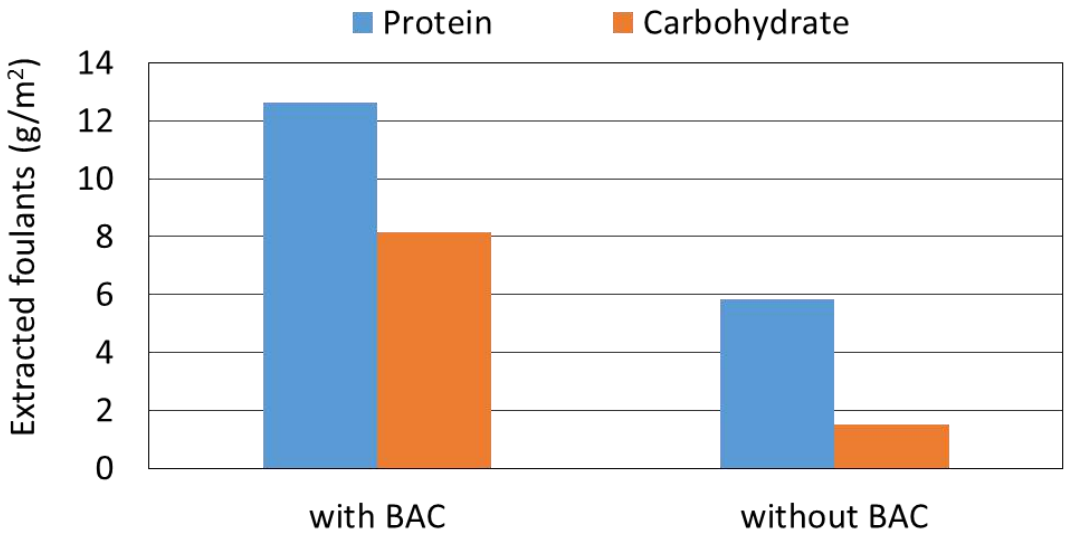


Figure 5.19 Protein and carbohydrate content in extracted foulants (The amounts of protein and carbohydrate were divided by effective area of ceramic membrane)

As shown in Figure 5.19, protein and carbohydrate content in ceramic membrane of w/ BAC were about 2 times and 4 times higher than that of w/o BAC, respectively. It can be assumed that the difference in protein and carbohydrate content was attributed to the increases of SMP like-materials after BAC or EPS produced by microorganisms forming biofouling.

In conclusion, BAC was able to control DBPs and their FP effectively, but it had potentially a negative effect on subsequent ceramic membrane filtration caused by the leakage of microorganisms or the formation of biofouling. Thus, BAC has a potential as one of options as additional treatment in case that DBPs should rigorously be controlled depending on the use of reclaimed water. However, a further study on efficient operation condition such as EBCT is needed to minimize the negative effect on ceramic membrane filtration by the addition of BAC.

5.4 Conclusions

In this chapter, DBPs and their FP were investigated in both O_3&CMF process for treating SE and PE to ensure public health. Moreover, the effect on not only the removal of DBPs but also ceramic membrane filtration caused by the addition of BAC treatment

to control DBPs rigidly were evaluated.

The following conclusions can be drawn:

1. Although a little amount of NDMA and TCM was formed, their FP was dramatically reduced during ozonation. However, ozonation formed primarily FAH up to a level of concentration which could be a problem on drinking water regulation established by Japan. It is necessary to control DBPs rigorously depending on the use of reclaimed water, and therefore the addition of BAC as treatment for the reduction of DBPs was considered.

2. It was expected that reclaimed water produced from PE by PACl+CMF+O_3 could contain both ADH and THMs at concentrations of several hundreds of μg/L. In addition, even though ozonation could reduce FP of NDMA, approximately 1000 ng/L of NDMA was remained in reclaimed water after chlorination. In case of PE, therefore, it is recommended that the utilization of reclaimed water should be restricted to the use which has less possibility to be exposed to users.

3. BAC could reduce DBPs examined in this study. Especially, FAH which is well known as easily biodegradable compounds was effectively removed through BAC. In addition, the extension of EBCT can improve the removal of both DBPs and their FP.

4. The leakage of microorganism such as general bacteria and heterotrophic bacteria from BAC was found, and moreover the increasing tendency of the peak intensity corresponding to SMP-like materials, which was considered as major foulants, was observed in EEM spectra. These phenomenon may cause accelerated membrane fouling. Indeed, not only higher peak intensity corresponding to SMP-like materials, but also greater protein and carbohydrate content were detected in extracted foulants from ceramic membrane of w/ BAC, compared to that of w/o BAC.

5. BAC has a potential as one of options as additional treatment in case that DBPs should rigorously be controlled depending on the use of reclaimed water. However, the optimization of operation condition such as EBCT is required to minimize the negative effect on ceramic membrane filtration by the addition of BAC.

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Chapter Ⅵ

Risk assessment of reclaimed water considering virus and disinfection by-product

6.1 Introduction

Virus removal and disinfection by-products (DBPs) formation during ozonation and ceramic membrane filtration combination process (O_3&CMF process) were investigated in Chapter IV and V. Although ozonation was effective to both virus removal and membrane fouling mitigation, it formed DBPs such as formaldehydes (FAH), chloroform (TCM) and *N*-nitrosodimethylamine (NDMA). It means that cancer risk caused by DBPs increased during ozonation while virus infection risk was reduced, indicating that there is a trade-off relationship between virus infection risk and DBPs cancer risk. Although virus infection risk (Masago et al., 2006; Barker et al., 2013) or DBPs cancer risk (Wang et al., 2007; Lee et al., 2009; Legay et al., 2013) has been reported in drinking water treatment system, there is only a few reports regarding the risk assessment of both virus and DBPs in water reclamation.

In this chapter, therefore, assessments of virus infection risk and cancer risk, caused by DBPs, were conducted depending on the purpose of reclaimed water. In addition, the trade-off relationship between virus infection risk and cancer risk was investigated.

6.2 Material and Methods

6.2.1 Procedure of risk assessment

Figure 6.1 illustrates the procedure for risk assessment.

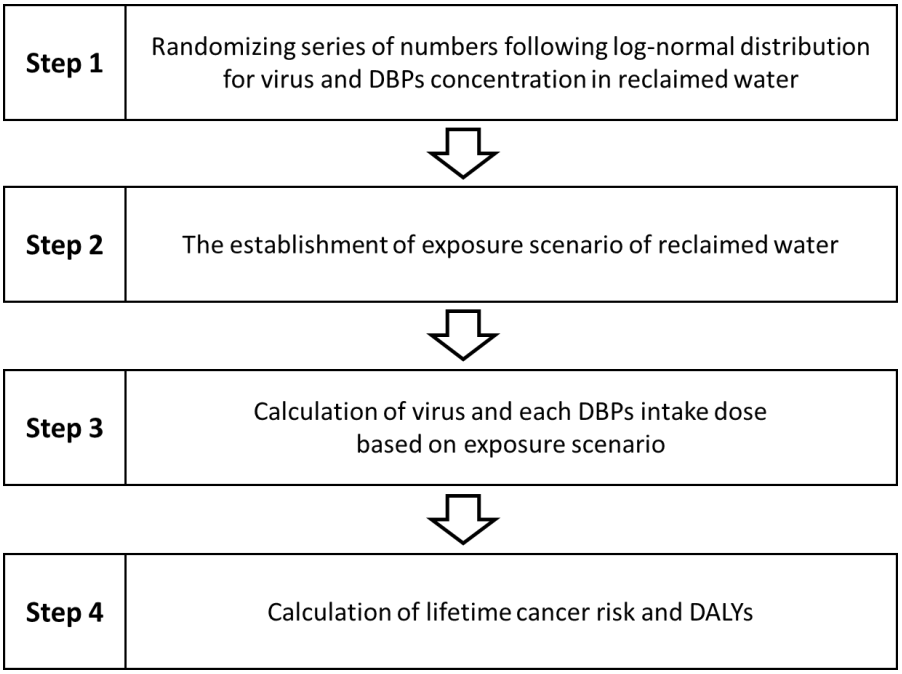
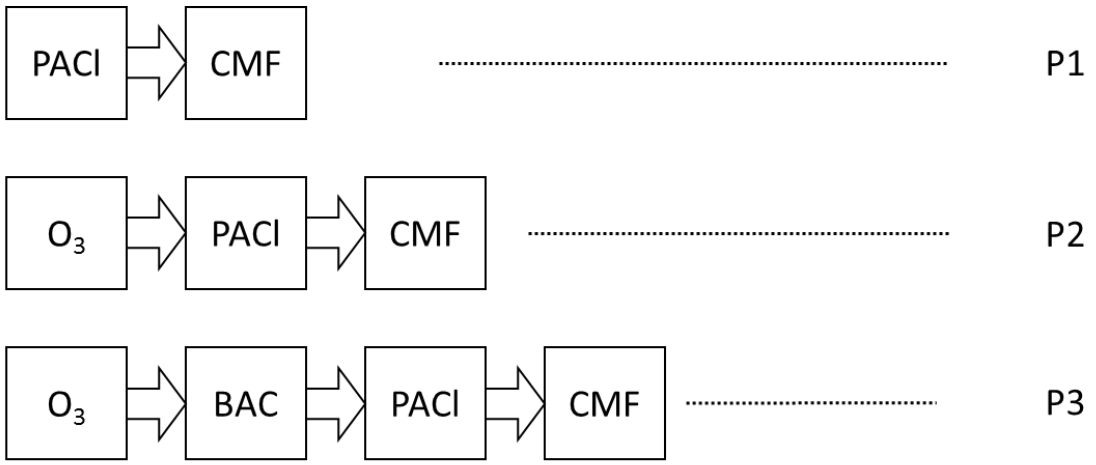


Figure 6.1 Schematic diagram of risk assessment

6.2.2 Treatment process for risk assessment

Among examined O_3&CMF process in Chapter IV and V, three treatment process was selected for risk assessment. Unfortunately, the data set of O_3&CMF process for treating primary effluent (PE) was insufficient to conduct probabilistic risk assessment. In this chapter, therefore, O_3&CMF process for treating secondary effluent (SE) was selected in priority.

Figure 6.2 illustrates schematic diagram of treatment process.



※ O_3, PACl, CMF and BAC indicate ozonation, coagulation, ceramic membrane filtration and biological activated carbon, respectively.

Figure 6.2 Schematic diagram of treatment process for risk assessment (P1, P2 and P3 represent abbreviations of each process)

The experimental set-up of each treatment process was explained in 4.2.4 and 5.2.5. The detail of operational condition was summarized in Table 6.1. Only P2 was divided into three cases (P2-2, P2-4 and P2-6) depending on the condition of ozone dosage. By comparing among these cases, the trade-off between virus removal and the formation of DBPs during ozonation was investigated.

Table 6.1 Operational condition of treatment process

Process	Abbreviation	Operational condition			
		O_3 (mg/L)	PACl (mg/L)	BAC (min)	CMF (m/d)
PACl+CMF	P1	0	25	-	4
O_3+PACl+CMF	P2-2	2	25	-	4
	P2-4	4	25	-	4
	P2-6	6	25	-	4
O_3+BAC+PACl+CMF	P3	6	25	3 ~ 5	4

6.2.3 Probabilistic risk assessment

The treatment variability of O_3&CMF process was determined as function of the

variability of virus or DBPs concentration in SE and the performance variability of unit treatment process (O_3, BAC and PACl+CMF). The data of virus concentration in SE were collected from the result of norovirus (NoV) concentration in Chapter VI (see Figure S1 in the supplementary material), and DBPs and their FP concentration was collected from the result in Chapter V. Virus or DBPs concentration in final product water was derived from a probability density function (PDF) of virus or DBPs concentration in SE and PDF of removal or formation during O_3&CMF process. According to previous researches, the distributions of many pollutants in wastewater treatment plant effluents follow a normal distribution or a log-normal distribution (Dean and Forsythe, 1976; Asano and Wassermann, 1979; Dean, 1981).

To evaluate treatment variability, the idea of assessing multiple barrier water treatment performance as a series of unit process performance PDFs was adapted in this study. The treatment performance of O_3&CMF process was assessed as a series of unit process performance. In addition, it has been reported that water treatment performances also follow a log-normal distribution (Olivieri et al., 1999). Therefore, it was assumed that treatment performance of O_3&CMF process was also fitted to log-normal PDFs.

PDF of virus or DBPs concentrations and treatment performance of O_3&CMF process was derived using Monte Carlo simulation. Monte Carlo simulation was run using Microsoft Excel (version. 2013). In order to predict virus or DBPs concentrations in final product water, a distribution type of input parameters such as mean and standard deviation of concentrations and removal (or DBPs formation) performance in O_3&CMF process was used. On basis of these input parameters, 10000 times of simulation were conducted in a Monte Carlo calculation, and the distributions of virus or DBPs concentrations in final product water was obtained. For the following exposure assessment, 5, 50 and 95th percentile values of virus or DBPs concentrations were adapted.

6.2.4 Exposure scenario

In this chapter, six exposure scenarios were assumed. Scenario 1 to 5 (recreational impoundment, municipal irrigation, garden irrigation, toilet flushing and crop irrigation) were already explained in chapter III. Moreover, scenario 6 (unintended IPR) was newly added in this chapter, because this purpose of reclaimed water could be one of the greatest threat to public health. Each exposure scenario was summarized in Table 6.2.

Table 6.2 Exposure scenarios

Scenario	Purpose	Risk receptor	Route of exposure	Exposure frequency/person/ year	Volume for single exposure	References
Scenario 1	Recreational impoundment	Swimmer	Accidental ingestion and dermal absorption	40	100	Tanaka et al., 1998
Scenario 2	Municipal irrigation	People involved	Ingestion	50	1	NRMMC et al., 2006
Scenario 3	Garden irrigation	Residents involved	Routine ingestion	100	1	NRMMC et al., 2006
Scenario 4	Toilet flushing	Residents involved	Ingestion of sprays	1100	0.01	NRMMC et al., 2006
Scenario 5	Crop irrigation	Consumer	Ingestion	140	1	NRMMC et al., 2006; WHO., 20016
Scenario 6	Unintended IPR	People involved	Ingestion, inhalation intake and dermal absorption	365	2000	USEPA., 1997; Wang., 2007

In scenario 6, it was assumed that reclaimed water is discharged into water supply source located upstream of a drinking water treatment plant, and virus and DBPs concentration in reclaimed water is supposed to be diluted to 50% level. In addition, virus and DBPs concentration in reclaimed water can be reduced in the environment depending on exposure scenarios, and DBPs is able to be formed during distribution systems due to chlorination for preventing microbial regrowth. Accordingly, virus and DBPs concentration in reclaimed water and in recycled water might be different. In this study, reclaimed water was defined as product water by O_3&CMF process, and recycled water was defined as water supplied in each scenario.

The concentration of virus and DBPs in recycled water was determined considering the fate of virus and DBPs in the environment, during distribution systems and drinking water treatment. It was summarized in Table 6.3 and 6.4 and explained in following 6.2.4.1 and 6.2.4.2.

6.2.4.1 Virus reduction in environmental and removal by drinking water treatment

In scenario 6, virus is supposed to be reduced in the environment, and be removed during drinking water treatment. According to Bae and Schwab (2008), the inactivation of MS2 was about 0.05 and 0.13 log/day in surface water at 4 and 25℃, respectively. While they dose not investigate the infectivity of NoV, viral RNA reduction of NoV was similar with that of MS2 (0.03 and 0.06 log/day for NoV and MS2 at 25℃, respectively). Thus, the reduction of virus in environment was assumed as 0.1 log/day (mean value of the reported MS2 inactivation in surface water).

Boudaud et al. (2012) reported that 4.75 log of MS2, 1.65 log of GA and 5.44 log of Qβ

was removed during conventional drinking water treatment process (coagulation, sedimentation and sand filtration). Meanwhile, Shirasaki et al. (2010) investigated the virus removal by coagulation and sand filtration using MS2, Qβ and recombinant NoV-like particles (rNV-VLPs), morphologically similar to native NoV, and as a result approximately 2 to 3 log of virus removal was obtained. According to a recent report (Asami et al., 2016), moreover, about 2 log of JC polyomavirus and PMMoV were removed in actual full scale drinking water treatment plant (coagulation, sedimentation and sand filtration). In this study, thus, it was assumed that 2 log of virus (minimum level of the reported removal rates) is removed during conventional drinking water treatment process. In addition, virus can be inactivated by chlorination. According to Cromeans et al. (2010), 2 log of murine NoV was inactivated after about 30 min contact time with 1 mg/L of monochloramine. Therefore, the inactivation of virus by chlorination was assumed as 2 log in this chapter. Consequently, it was assumed that total 4 log of virus could be removed during drinking water treatment process. In scenario 1 to 5, on the other hand, virus concentration is supposed to be no change during distribution system.

6.2.4.2 The calculation of virus concentration in recycled water

Based on the exposure scenario, virus concentration in recycled water was calculated as follows.

$$C_{w(V)} = C_{re(V)} \times Di \times \left(1 - R_{e(V)}\right) \times \left(1 - R_{d(V)}\right) \quad \text{(E.q 6.1)}$$

where,

C_w is concentration of virus in recycled water

C_{re} is concentration of virus in reclaimed water

R_e is reduction rate in the environment

R_d is removal rate during drinking water treatment process

Di is dilution rate

Index *(V)* is virus

6.2.4.3 DBPs reduction in environmental and formation during distribution systems

According to a previous report, more than 10 ng/L of NDMA was formed from about 50 ng/L of NDMA precursor concentration after chloramination under typical drinking water

treatment conditions (Gerecke and Sedlak, 2003). Moreover, there was a report that formation potential of NDMA was assessed in treatment systems used for landscape irrigation in which chloramines served as disinfectant (Pehlivanoglu-Mantas et al., 2006a). They estimated that approximately 20% of the NDMA precursors were converted into NDMA in investigated treatment systems. Meanwhile, there was a report that mean thrihalomethanes (THMs) concentration increased about 20% during distribution system (Chen et al., 1998). In scenario 1 to 6, therefore, it was assumed that approximately 20% of NDMA or TCM precursors is converted into NDMA or TCM, respectively. On the other hand, it was assumed that FAH is not formed by chlorination.

In addition, DBPs can be reduced in the environment in scenario 6. Each DBP reduction rate in the environment was decided from references, and it was summarized in Table 6.3 and 6.4. It was well documented that the degradation of FAH, NDMA and TCM in aquatic environments was mainly caused by biodegradation, photolysis and volatilization, respectively (Smith and Bomberger, 1980; Gerike and Gode, 1990; Eiroa et al., 2004; Plumlee et al., 2007). According to a report of NDMA photolysis in a river, NDMA residual rate in a river was 61% as 95th percentile during 24 hours of travel time (Pehlivanoglu-Mantas et al., 2006a). In this study, the travel time of the discharged reclaimed water was assumed as 24 hours, and NDMA reduction rate by photolysis was set to be 39% in scenario 6. In case of FAH and TCM reduction in the environment, it was decided by reference to a literature reporting DBPs fate in a river (Itai, 2016). According to this report, the reduction of FAH and TCM in the river was evaluated by the exponential decay and their decay constant was 0.207 and 0.176 h^{-1}, respectively.

On the other hand, NDMA and TCM FP reduction in the environment in scenario 6 was supposed to be negligible. According to Pehlivanoglu-Mantas et al. (2006b), wastewater-derived NDMA precursors remained elevated over the 40km reach downstream of the wastewater outfalls, which corresponds to a hydraulic retention time of approximately 40h.

In addition, there is a report that THM precursors were unchanged in a river because they were related with non-biodegradable organic matter (Chen et al., 2009).

Table 6.3 Reduction and formation of virus and DBPs in the environment or during distribution system for each exposure scenario

Scenario	Common	Virus		DBPs					
	Dilution	Reduction in the environment	Removal by drinking water treatment	Reduction in the environment			Formation during distribution system		
				FAH	NDMA	TCM	FAH	NDMA	TCM
Scenario 1	-	-	-	-	-	-	-	20% of FP	20% of FP
Scenario 2	-	-	-	-	-	-	-	20% of FP	20% of FP
Scenario 3	-	-	-	-	-	-	-	20% of FP	20% of FP
Scenario 4	-	-	-	-	-	-	-	20% of FP	20% of FP
Scenario 5	-	-	-	-	-	-	-	20% of FP	20% of FP
Scenario 6	50%	0.1 log/day	4 log	See Table 6.4	See Table 6.4	See Table 6.4	-	20% of FP	20% of FP

Table 6.4 Reduction of DBPs in the environment

Parameters	DBPs		
	FAH	NDMA	TCM
Major cause of reduction	Biodegradation	Photodegradation	Volatilization
Degradation rate (%) or Exponential decay constant (h^{-1})	0.207[a]	31%	0.176[a]

[a] FAH and TCM was assumed to be degraded in accordance with exponential decay in environment

Based on the exposure scenario, each DBPs concentration in recycled water was calculated as follows.

$$C_{w(D)} = C_{re(V)} \times Di \times \left(1 - R_{e(D)}\right) + C_{re(FP)} \times Di \times F \qquad \text{(E.q 6.2)}$$

where

C_w is concentration of DBPs or DBPs FP in recycled water

C_{re} is concentration of DBPs or DBPs FP in reclaimed water

R_e is reduction rate in the environment

Di is dilution rate

F is DBPs formation rate

Index *(D)* is DBPs

Index (*FP*) is DBPs FP

6.2.4.4 The calculation of chronic daily DBPs intake

On the basis of each DBPs concentration in recycled water, the amount of chronic daily DBP intake was calculated. Table 6.5 and 6.6 shows input parameters used for calculation.

Table 6.5 Common input parameters for each exposure scenarios.

Input parameters	Unit	Values					
		Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
		Recreational impoundment	Municipal irrigation	Garden irrigation	Toilet flushing	Crop irrigation	Unintended IPR
Oral ingestion							
DBPs in water (Cw)	μg/L or ng/L	See Table 6.15 ~ 6.17	See Table 6.15 ~ 6.17	See Table 6.15 ~ 6.17	See Table 6.15 ~ 6.17	See Table 6.15 ~ 6.17	See Table 6.15 ~ 6.17
Ingestion rate (IR)	L/day	-	-	-	-	-	2
	L/single exposure	0.1	0.001	0.001	0.00001	0.001	-
Exposure frequency (EF)	day/year	-	-	-	-	-	365
	exposure time/year	40	50	100	1100	140	-
Exposure duration (ED)	year	29	29	29	29	29	29
Conversion factor (CF)	mg/ng or mg/μg	10^{-6} or 10^{-3}	10^{-6} or 10^{-3}	10^{-6} or 10^{-3}	10^{-6} or 10^{-3}	10^{-6} or 10^{-3}	10^{-6} or 10^{-3}
Body weight (BW)	kg	70	70	70	70	70	70
Average time	day	70 × 365	70 × 365	70 × 365	70 × 365	70 × 365	70 × 365
inhalation in shower							
DBPs in air (Cair)	ng/L	-	-	-	-	-	See Table 6.15 ~ 6.17
Ventilation rate (VR)	m^3/h	-	-	-	-	-	15
Absorption efficiency in alveoli (AE)		-	-	-	-	-	50%
Room volume (V)	m^3	-	-	-	-	-	10
Water flow rate (QL)	L/min	-	-	-	-	-	5
Air flow rate (QG)	L/min	-	-	-	-	-	50
Water temperature (T)	℃	-	-	-	-	-	44
Dimensionless Henry's law canstants (H)		-	-	-	-	-	See Table 6.6
Overall mass transfer coefficient ($K_{OL}A$)		-	-	-	-	-	See Table 6.6
Exposure time (ET)	min/day	-	-	-	-	-	18.9
Exposure frequency (EF)	day/year	-	-	-	-	-	365
Exposure duration (ED)	year	-	-	-	-	-	29
Conversion factor (CF)	mg/ng or mg/μg	-	-	-	-	-	10^{-6} or 10^{-3}
Body weight (BW)	kg	-	-	-	-	-	70
Average time	day	-	-	-	-	-	70 × 365
Dermal absorption							
Skin surface area (SA)	m^2	1.79375	-	-	-	-	1.79375
Fraction of skin in contact with water (F)		0.8	-	-	-	-	0.8
Permeability coefficient (PC)	cm/h	0.000251	-	-	-	-	0.000251
Exposure time (ET)	min/day	18.9	-	-	-	-	18.9
Exposure frequency (EF)	day/year	40	-	-	-	-	365
Exposure duration (ED)	year	29	29	29	29	29	29
Conversion factor (CF)	mg/ng or mg/μg	10^{-6} or 10^{-3}	10^{-6} or 10^{-3}	10^{-6} or 10^{-3}	10^{-6} or 10^{-3}	10^{-6} or 10^{-3}	10^{-6} or 10^{-3}
Body weight (BW)	kg	70	70	70	70	70	70
Average time	day	70 × 365	70 × 365	70 × 365	70 × 365	70 × 365	70 × 365

Table 6.6 Input parameters of each DBPs for exposure scenarios.

Parameters	Unit	DBPs		
		FAH	NDMA	TCM
Dimensionless Henry's law constant (H)		1.38×10^{-5} (RAIS)	7.44×10^{-5} (RAIS)	0.15 (RAIS)
Overall mass transfer coefficient ($K_{OL}A$)	L/min	1.52×10^{-9} [a]	1.52×10^{-9} [b]	7.4 (Little et al., 1992)

Parenthesis indicates references

[a] According to Wang et al. (2007), the $K_{OL}A$ of FAH was calculated based on following formula : $1/K_{OL} = 1/(H^* K_G)+1/ K_L$, where H is the Henry's constant, K_G and K_L represent the gas- and liquid- film mass transfer coefficient of FAH

[b] The $K_{OL}A$ value of FAH was used for the $K_{OL}A$ of NDMA

An exposure assessment was conducted to evaluate the DBPs uptake via oral ingestion, inhalation and dermal absorption. For inhalation, exposure during shower was assumed as the major exposure route, while inhalation exposure to DBPs in cooking was not considered in this study. Inhalation intake of DBPs volatilized from water into the shower room was calculated using inhalation exposure model, which was developed based on two-resistance theory proposed by Little et al. (1992).

The equations for calculation of chronic daily intakes are shown as follows:

$$\text{Oral Ingestion (mg/kg} - \text{day)} = [C_w \times \text{IR} \times \text{EF} \times \text{ED} \times \text{CF}]/[\text{BW} \times \text{AT}] \quad \text{(E.q 6.3)}$$

$$\text{Dermal absorption (mg/kg} - \text{day)} =$$

$$[C_w \times \text{SA} \times \text{F} \times \text{PC} \times \text{ET} \times \text{EF} \times \text{ED} \times \text{CF}]/[\text{BW} \times \text{AT}] \quad \text{(E.q 6.4)}$$

$$\text{Inhalation Intake (mg/kg} - \text{day)} =$$

$$[C_{air} \times \text{VR} \times \text{AE} \times \text{ET} \times \text{EF} \times \text{ED} \times \text{CF}]/[\text{BW} \times \text{AT}] \quad \text{(E.q 6.5)}$$

For inhalation intake, C_{air} is calculated by:

$$\text{C}_{\text{air}} = (Y_s(t) + Y_{si})/2, \quad \text{(E.q 6.6)}$$

where

Y_{si} is the initial DBPs concentration in the shower room (assumed as 0 ng/L)

$Y_s(t)$ is the DBPs concentration in the shower room at time t (min).

And

$$Y_s(t) = [1 - exp(-bt)](a/b) \quad \text{(E.q 6.7)}$$

$$b = \{(Q_L/H)\}[1 - \exp(-N)] + Q_G/V_s \quad \text{(E.q 6.8)}$$

$$a = \{Q_L C_w[1 - \exp(-N)]\}/V_s \quad \text{(E.q 6.9)}$$

$$N = (K_{OL}A)/Q_L \quad \text{(E.q 6.10)}$$

where *N* is a dimensionless coefficient that calculated from $K_{OL}A$

Detailed description for the parameters and the input values can be seen elsewhere (Wang et al., 2007).

6.2.5 Risk Calculations

6.2.5.1 Disability adjusted life year calculation

Disability adjusted life year (DALY) was calculated in accordance with the method described in 3.2.6

6.2.5.2 Lifetime cancer risk of DBPs

The lifetime cancer risk of DBPs was calculated as follows.

$$\text{Cancer risk} = \text{Lifetime daily DBPs intake} \times \text{DBPs slope factor} \quad \text{(E.q 6.11)}$$

$$\text{Total exposure cancer risk} = \text{Risk}_{\text{oral ingestion}} + \text{Risk}_{\text{inhalation}} + \text{Risk}_{\text{dermal adsorption}} \quad \text{(E.q 6.12)}$$

The slope factor of each DBP was collected from Integrated Risk Information System (IRIS) and Risk Assessment Information System (RAIS). For the slope factor of dermal absorption, which is not available in IRIS and RAIS, it is assumed to be equal to the slope factor for inhalation. The slope factor of each DBP via different exposure routes was summarized in Table 6.7.

Table 6.7 Slope factor of each DBPs

Slope factor (SF) [(mg/kg-day)$^{-1}$]	DBPs		
	FAH	NDMA	TCM
Oral ingestion	2.1×10^{-2} (OEHHA)	5.1×10^{1} (RAIS)	3.1×10^{-2} (RAIS)
Inhalation intake	2.1×10^{-2} (RAIS)	1.6×10^{1} (RAIS)	1.9×10^{-2} (RAIS)
Dermal absorption	2.1×10^{-2} [a]	1.6×10^{1} [a]	1.9×10^{-2} [a]

Parenthesis indicates references

[a] The SF of inhalation intake was used for the SF of dermal absorption

6.3 Results and discussion

6.3.1 Risk assessment of virus

6.3.1.1 Estimated virus concentration in reclaimed water

The PDF of virus concentration in reclaimed water was derived through 10,000 times Monte Carlo simulation. Mean virus concentration in SE and mean virus removal rate by ozonation and PACl+CMF, and their standard deviation was used for the estimation of virus concentration in reclaimed water. Input parameters for the log-normal distribution of virus concentration and removal was summarized in Table 6.8. Virus concentration in recycled water for each scenario was calculated using the 5th, 50th and 95th percentile concentration of virus in reclaimed water. The estimated virus concentration in reclaimed water and in recycled water for each scenario was summarized in Table 6.9 and Table 6.10, respectively.

In recycled water produced by P1, 1.7×10^{-6} to 1.8×10^{-3} copies/L of virus concentration was obtained for scenario 1 to 5, while 6.7×10^{-13} to 7.1×10^{-10} copies/L was obtained for scenario 6. In recycled water produced by P2, 4.3×10^{-11} to 8.8×10^{-8} copies/L of virus concentration was obtained for scenario 1 to 5, whereas 1.7×10^{-17} to 3.5×10^{-14} copies/L was obtained for scenario 6. It was possible to decrease virus concentration in recycled water by incorporating ozonation. However, there was no significant difference in virus

concentration in recycled water with increasing ozone dosage.

Table 6.8 Input parameters for the log-normal distribution of virus concentration and virus removal rate

Input Parameters	Virus concentration in SE (copies/L)	Virus removal rate (log)			
		PACl+CMF (P1)	O_3+PACl+CMF (P2)		
			2 mg-O_3/L	4 mg-O_3/L	6 mg-O_3/L
Distribution			Log-normal		
Number of samples	9	4	5	4	5
Arithmatic mean of C (copies/L)	$3.2x10^4$				
Standard deviation of C	$3.5x10^4$				
Arithmatic mean of R (log)		8.4	4.9 (O_3) 8.1 (PACl+CMF)	5.7 (O_3) 6.8 (PACl+CMF)	6.1 (O_3) 6.8 (PACl+CMF)
Standard deviation of R		0.7	0.5 (O_3) 0.3 (PACl+CMF)	0.3 (O_3) 0.4 (PACl+CMF)	0.6 (O_3) 0.5 (PACl+CMF)

C : concentration, R : removal

Table 6.9 Estimated virus concentration in reclaimed water

Estimated virus concentration In reclaimed water (copies/L)		SE	Reclaimed water			
			PACl+CMF (P1)	O_3+PACl+CMF (P2)		
				2 mg-O_3/L	4 mg-O_3/L	6 mg-O_3/L
Estimated concentration in given percentile	5%	$1.6x10^3$	$1.7x10^{-6}$	$6.0x10^{-11}$	$1.9x10^{-10}$	$4.3x10^{-11}$
	50%	$9.6x10^4$	$6.3x10^{-5}$	$1.8x10^{-9}$	$4.8x10^{-9}$	$1.7x10^{-9}$
	95%	$1.4x10^5$	$1.8x10^{-3}$	$4.0x10^{-8}$	$8.8x10^{-8}$	$7.5x10^{-8}$

Table 6.10 Estimated virus concentration in recycled water for each scenario

	PACl+CMF (P1) (copies/L)			O_3+PACl+CMF (P2) (copies/L)								
				P2-2			P2-4			P2-6		
	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%
Scenario 1	$1.7x10^{-6}$	$6.3x10^{-5}$	$1.8x10^{-3}$	$6.0x10^{-11}$	$1.8x10^{-9}$	$4.0x10^{-8}$	$1.9x10^{-10}$	$4.8x10^{-9}$	$8.8x10^{-8}$	$4.3x10^{-11}$	$1.7x10^{-9}$	$7.5x10^{-8}$
Scenario 2	$1.7x10^{-6}$	$6.3x10^{-5}$	$1.8x10^{-3}$	$6.0x10^{-11}$	$1.8x10^{-9}$	$4.0x10^{-8}$	$1.9x10^{-10}$	$4.8x10^{-9}$	$8.8x10^{-8}$	$4.3x10^{-11}$	$1.7x10^{-9}$	$7.5x10^{-8}$
Scenario 3	$1.7x10^{-6}$	$6.3x10^{-5}$	$1.8x10^{-3}$	$6.0x10^{-11}$	$1.8x10^{-9}$	$4.0x10^{-8}$	$1.9x10^{-10}$	$4.8x10^{-9}$	$8.8x10^{-8}$	$4.3x10^{-11}$	$1.7x10^{-9}$	$7.5x10^{-8}$
Scenario 4	$1.7x10^{-6}$	$6.3x10^{-5}$	$1.8x10^{-3}$	$6.0x10^{-11}$	$1.8x10^{-9}$	$4.0x10^{-8}$	$1.9x10^{-10}$	$4.8x10^{-9}$	$8.8x10^{-8}$	$4.3x10^{-11}$	$1.7x10^{-9}$	$7.5x10^{-8}$
Scenario 5	$1.7x10^{-6}$	$6.3x10^{-5}$	$1.8x10^{-3}$	$6.0x10^{-11}$	$1.8x10^{-9}$	$4.0x10^{-8}$	$1.9x10^{-10}$	$4.8x10^{-9}$	$8.8x10^{-8}$	$4.3x10^{-11}$	$1.7x10^{-9}$	$7.5x10^{-8}$
Scenario 6	$6.7x10^{-13}$	$2.5x10^{-11}$	$7.1x10^{-10}$	$2.4x10^{-17}$	$7.0x10^{-16}$	$1.6x10^{-14}$	$7.7x10^{-17}$	$1.9x10^{-15}$	$3.5x10^{-14}$	$1.7x10^{-17}$	$6.6x10^{-16}$	$3.0x10^{-14}$

6.3.1.2 Estimated risk of virus infection

On basis of virus concentration in recycled water and exposure scenario, DALYs was calculated (see Table S3 in the supplementary material). Figure 6.3 shows DALY/person/years ($DALY_{pppy}$) for each scenario.

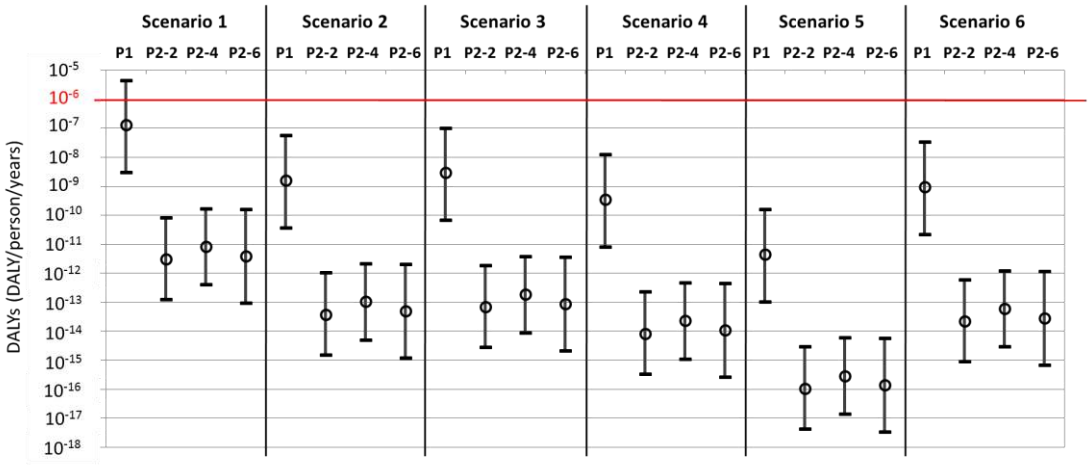


Figure 6.3 $DALY_{pppy}$ for exposure scenario

Virus infection risk from using recycled water produced by P1 ranged in $3.7x10^{-6}$ to $1.2x10^{-13}$ $DALY_{pppy}$. These virus infection risks met acceptable risk set by WHO of 10^{-6} $DALY_{pppy}$ in scenario 2 to 6, but the 95th percentile virus infection risk was higher than the acceptable risk in scenario 1. It indicated that P1 was insufficient as treatment process when the use of reclaimed water was recreational impoundment (scenario 1).

By incorporating ozonation, however, virus infection risk decreased by 10^{-4} to 10^{-6} $DALY_{pppy}$, and as a result, infection risk due to exposure to viruses in recycled water produced by P2 met acceptable risk in all exposure scenario. Moreover, there was no significant difference between infection risk from exposure to recycled water produced by P2-2, P2-4 and P2-6, because there was no significant difference between virus concentration in reclaimed water, as described in Table 6.8.

6.3.2 Lifetime cancer risk assessment of DBPs

6.3.2.1 Estimated DBPs concentration in reclaimed water

The PDF of DBPs concentration in reclaimed water was also derived through 10,000 times Monte Carlo simulation. Each DBP concentration in reclaimed water was estimated using mean and standard deviation value of both DBPs concentration in SE and formation or removal of DBPs during O_3&CMF process. Input parameters for the log-normal distribution of DBPs concentration and removal or formation was summarized in Table 6.11 to 6.13. Because PACl+CMF could not remove DBPs, as mentioned in Chapter V, DBPs concentration in reclaimed water produced by P1 was assumed as the same with that in SE. DBPs concentration increased during ozonation, and decreased by BAC. The concentration of NDMA FP and TCM FP decreased by both ozonation and BAC. The estimated DBPs concentration in reclaimed water was summarized in Table 6.14 to Table 6.16, and the estimated DBP concentration in recycled water and air was summarized in Table 6.17 to 6.19.

Table 6.11 Input parameters for the log-normal distribution of FAH concentration and removal or formation

Input Parameters	FAH concentration in SE (μg/L)	Formation or removal during O_3&CMF process (%)				
		PACl+CMF (P1)	O_3+PACl+CMF (P2)			O_3+BAC+PACl+CMF(P3)
			2 mg-O_3/L	4 mg-O_3/L	6 mg-O_3/L	6 mg-O_3/L
Distribution		Log-normal				
Number of samples	8	8	4	4	6	6
Arithmatic mean of C (μg/L)	11.82					
Standard deviation of C	9.54					
Arithmatic mean of F or R (%)		0	+334.96	+585.38	+874.50	-81.50
Standard deviation of F or R		0	193.51	101.65	434.47	7.56

C : concentration, F : formation, R : removal
+, - means DBPs formation and removal by treatment, respectively

Table 6.12 Input parameters for the log-normal distribution of NDMA and NDMA FP concentration and formation or removal

Input Parameters		NDMA or NDMA FP concentration in SE (ng/L)	Formation or removal during O_3&CMF process (%)				
			PACl+CMF (P1)	O_3+PACl+CMF (P2)			O_3+BAC+PACl+CMF(P3)
				2 mg-O_3/L	4 mg-O_3/L	6 mg-O_3/L	6 mg-O_3/L
	Distribution		Log-normal				
	Number of samples	8	8	3	3	6	4
NDMA	Arithmatic mean of C (ng/L)	4.57					
	Standard deviation of C	3.16					
	Arithmatic mean of F or R (%)		0	+232.72	+234.50	+233.64	-48.35
	Standard deviation of F or R		0	204.89	108.17	58.43	14.24
NDMA FP	Arithmatic mean of C (ng/L)	90.92					
	Standard deviation of C	33.02					
	Arithmatic mean of F or R (%)		0	-70.91	-72.20	-62.87	-14.82
	Standard deviation of F or R		0	6.46	6.47	17.24	12.83

C : concentration, F : formation, R : removal
+, - means DBPs formation and removal by treatment, respectively

Table 6.13 Input parameters for the log-normal distribution of TCM and TCM FP concentration and formation or removal

Input Parameters		TCM or TCM FP concentration in SE (μg/L)	Formation or removal during O_3&CMF process (%)				
			PACl+CMF (P1)	O_3+PACl+CMF (P2)			O_3+BAC+PACl+CMF(P3)
				2 mg-O_3/L	4 mg-O_3/L	6 mg-O_3/L	6 mg-O_3/L
	Distribution		Log-normal				
	Number of samples	8	8	3	3	6	4
TCM	Arithmatic mean of C (μg/L)	0.53					
	Standard deviation of C	0.16					
	Arithmatic mean of F or R (%)		0	+19.27	+19.86	+122.42	-47.83
	Standard deviation of F or R		0	16.65	4.73	180.50	8.70
TCM FP	Arithmatic mean of C (μg/L)	30.27					
	Standard deviation of C	9.09					
	Arithmatic mean of F or R (%)		0	-35.78	-47.21	-29.00	-14.82
	Standard deviation of F or R		0	8.20	42.08	24.86	12.83

C : concentration, F : formation, R : removal
+, - means DBPs formation and removal by treatment, respectively

Table 6.14 Estimated FAH concentration in reclaimed water

Estimated FAH concentration (μg/L)		SE	Reclaimed water				
			PACl+CMF (P1)	O_3+PACl+CMF (P2)			O_3+BAC+PACl+CMF (P3)
				2 mg-O_3/L	4 mg-O_3/L	6 mg-O_3/L	6 mg-O_3/L
Estimated concentration in given percentile	5%	3.92	3.92	12.95	26.06	29.32	2.81
	50%	11.80	11.80	46.31	79.81	105.48	18.31
	95%	34.26	34.26	190.02	239.31	390.46	78.64

Table 6.15 Estimated NDMA and NDMA FP concentration in reclaimed water

Estimated concentration (ng/L)			SE	Reclaimed water				
				PACl+CMF (P1)	O_3+PACl+CMF (P2)			O_3+BAC+PACl+CMF (P3)
					2 mg-O_3/L	4 mg-O_3/L	6 mg-O_3/L	6 mg-O_3/L
NDMA	Estimated concentration in given percentile	5%	1.43	1.43	2.89	4.08	4.41	0.47
		50%	4.57	4.57	13.75	14.23	14.95	1.38
		95%	14.59	14.59	77.81	54.43	52.07	2.32
NDMA FP	Estimated concentration in given percentile	5%	57.79	57.79	13.77	12.62	1.97	0.60
		50%	90.98	90.98	26.17	24.93	33.62	27.78
		95%	144.71	144.71	46.41	44.74	69.15	59.74

Table 6.16 Estimated TCM and TCM FP concentration in reclaimed water

Estimated concentration (μg/L)			SE	Reclaimed water				
				PACl+CMF (P1)	O_3+PACl+CMF (P2)			O_3+BAC+PACl+CMF (P3)
					2 mg-O_3/L	4 mg-O_3/L	6 mg-O_3/L	6 mg-O_3/L
TCM	Estimated concentration in given percentile	5%	0.34	0.34	0.37	0.41	0.43	0.20
		50%	0.53	0.53	0.63	0.69	0.82	0.43
		95%	0.81	0.81	3.63	0.98	5.11	2.65
TCM FP	Estimated concentration in given percentile	5%	20.10	20.10	11.89	3.82	0.07	0.11
		50%	30.42	30.42	19.36	20.09	22.06	18.26
		95%	45.50	45.50	30.56	33.56	37.14	21.52

In scenario 1 to 5, FAH, NDMA and TCM concentration in recycled water produced by P1 were 3.92 to 34.26 μg/L, 12.99 to 43.53 ng/L and 4.36 to 9.91 μg/L, respectively. As same with the result in Chapter V, these DBPs concentration in recycled water increased by incorporating ozonation (P2), and decreased by BAC (P3). Consequently, FAH, NDMA and TCM concentration in recycled water produced by P2 were 12.95 to 390.46 μg/L, 4.80 to 87.09 ng/L and 0.44 to 12.54 μg/L, respectively. In addition, FAH, NDMA and TCM concentration in recycled water produced by P3 were 2.81 to 78.64 μg/L, 0.59 to 14.27 ng/L and 0.22 to 6.95 μg/L, respectively.

In scenario 6, FAH, NDMA and TCM concentration in recycled water were 0.01 to 1.36 μg/L, 0.15 to 28.22 ng/L, 0.01 to 4.55 μg/L, respectively. The degradation of FAH and TCM in the environment was relatively larger than NDMA. TCM concentration in air was 0 to 0.12 μg/L, and FAH and NDMA were calculated to be negligible because they were not highly-volatile.

Table 6.17 Estimated FAH concentration in recycled water and air for each exposure scenario

	PACl+CMF (P1) (μg/L)			O_3+PACl+CMF (P2) (μg/L)									O_3+BAC+PACl+CMF (P3) (μg/L)		
				P2-2			P2-4			P2-6					
	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%
Scenario 1	3.92	11.80	34.26	12.95	46.31	190.02	26.06	79.81	239.31	29.32	105.48	390.46	2.81	18.31	78.64
Scenario 2	3.92	11.80	34.26	12.95	46.31	190.02	26.06	79.81	239.31	29.32	105.48	390.46	2.81	18.31	78.64
Scenario 3	3.92	11.80	34.26	12.95	46.31	190.02	26.06	79.81	239.31	29.32	105.48	390.46	2.81	18.31	78.64
Scenario 4	3.92	11.80	34.26	12.95	46.31	190.02	26.06	79.81	239.31	29.32	105.48	390.46	2.81	18.31	78.64
Scenario 5	3.92	11.80	34.26	12.95	46.31	190.02	26.06	79.81	239.31	29.32	105.48	390.46	2.81	18.31	78.64
Scenario 6 C_w	0.01	0.04	0.12	0.05	0.16	0.66	0.09	0.28	0.83	0.10	0.37	1.36	0.01	0.06	0.27
Scenario 6 C_{air}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 6.18 Estimated NDMA concentration in recycled water and air for each exposure scenario

	PACl+CMF (P1) (ng/L)			O_3+PACl+CMF (P2) (ng/L)									O_3+BAC+PACl+CMF (P3) (ng/L)		
				P2-2			P2-4			P2-6					
	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%
Scenario 1	12.99	22.77	43.53	5.64	18.98	87.09	4.20	19.79	66.38	4.80	21.67	65.90	0.59	6.94	14.27
Scenario 2	12.99	22.77	43.53	5.64	18.98	87.09	4.20	19.79	66.38	4.80	21.67	65.90	0.59	6.94	14.27
Scenario 3	12.99	22.77	43.53	5.64	18.98	87.09	4.20	19.79	66.38	4.80	21.67	65.90	0.59	6.94	14.27
Scenario 4	12.99	22.77	43.53	5.64	18.98	87.09	4.20	19.79	66.38	4.80	21.67	65.90	0.59	6.94	14.27
Scenario 5	12.99	22.77	43.53	5.64	18.98	87.09	4.20	19.79	66.38	4.80	21.67	65.90	0.59	6.94	14.27
Scenario 6 C_w	6.22	10.57	19.08	2.25	6.82	28.22	1.61	7.14	22.25	1.61	8.99	24.67	0.15	3.37	7.42
Scenario 6 C_{air}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

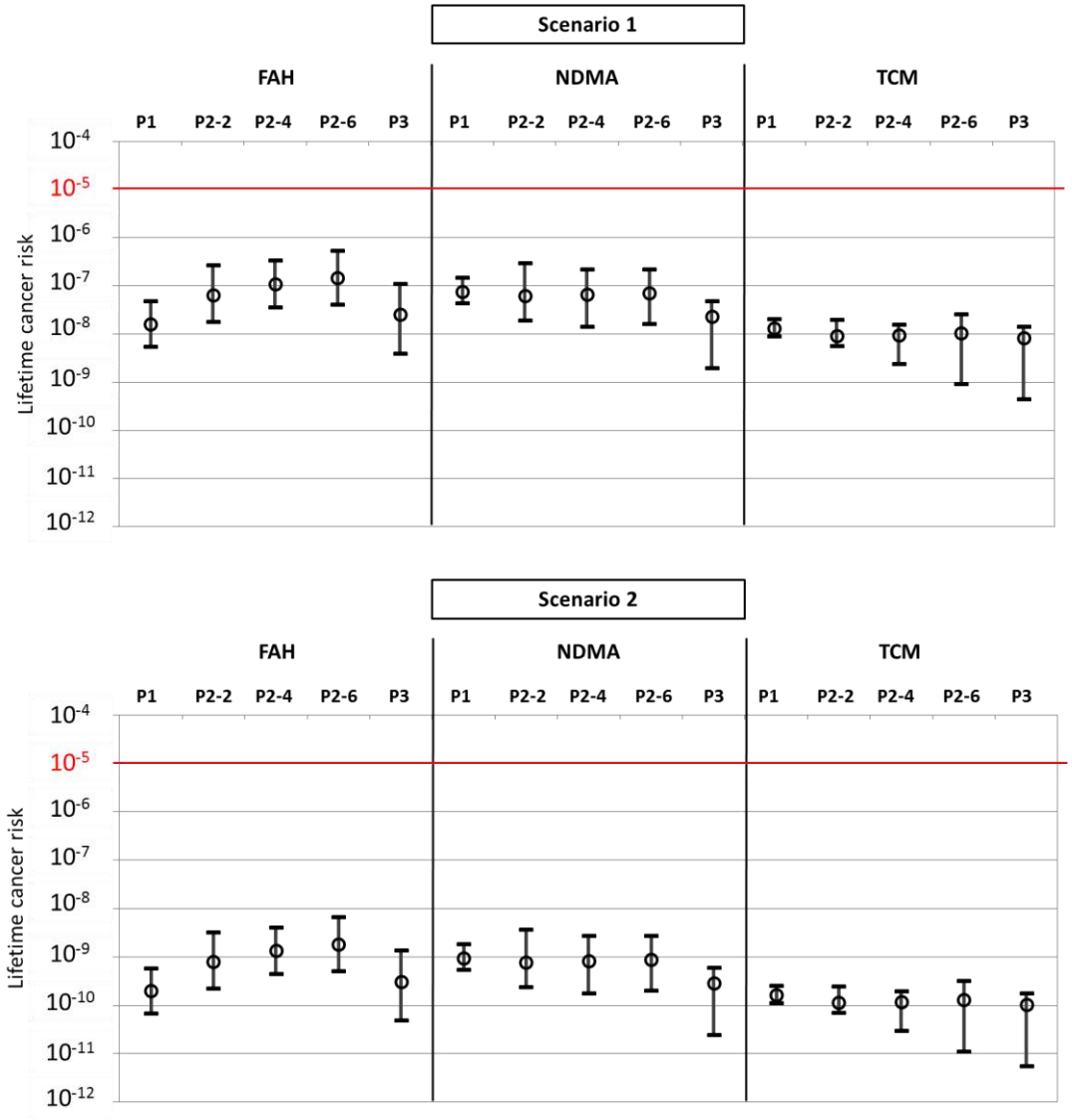
Table 6.19 Estimated TCM concentration in recycled water and air for each exposure scenario

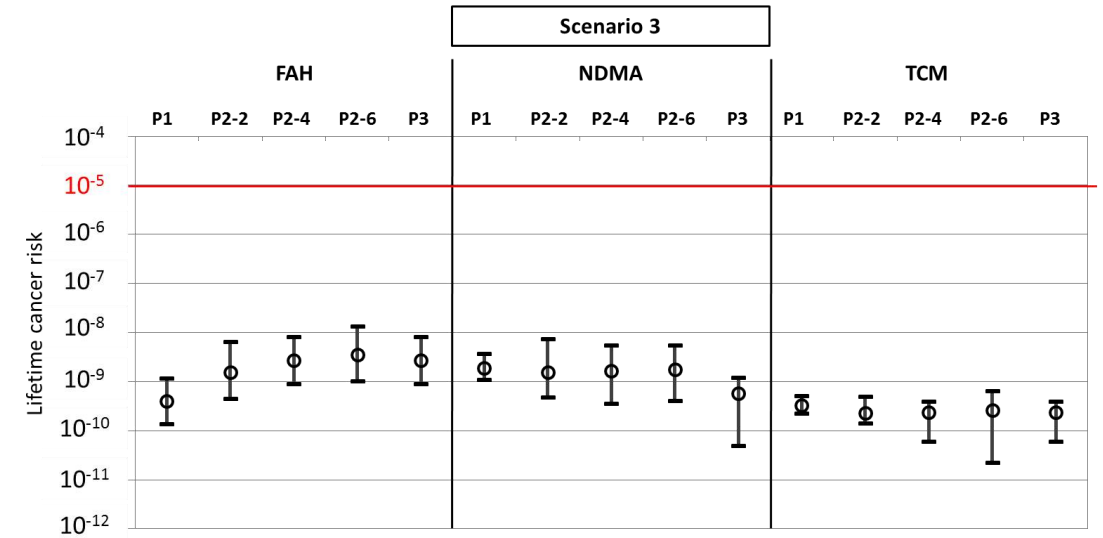
	PACl+CMF (P1) (μg/L)			O_3+PACl+CMF (P2) (μg/L)									O_3+BAC+PACl+CMF (P3) (μg/L)		
				P2-2			P2-4			P2-6					
	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%
Scenario 1	4.36	6.61	9.91	2.75	4.50	9.74	1.17	4.71	7.69	0.44	5.23	12.54	0.22	4.08	6.95
Scenario 2	4.36	6.61	9.91	2.75	4.50	9.74	1.17	4.71	7.69	0.44	5.23	12.54	0.22	4.08	6.95
Scenario 3	4.36	6.61	9.91	2.75	4.50	9.74	1.17	4.71	7.69	0.44	5.23	12.54	0.22	4.08	6.95
Scenario 4	4.36	6.61	9.91	2.75	4.50	9.74	1.17	4.71	7.69	0.44	5.23	12.54	0.22	4.08	6.95
Scenario 5	4.36	6.61	9.91	2.75	4.50	9.74	1.17	4.71	7.69	0.44	5.23	12.54	0.22	4.08	6.95
Scenario 6 C_w	1.99	2.99	4.55	0.97	1.92	3.17	0.39	2.01	3.36	0.15	2.22	3.76	0.01	1.17	2.17
Scenario 6 C_{air}	0.05	0.08	0.12	0.02	0.05	0.08	0.01	0.05	0.09	0.00	0.06	0.10	0.00	0.03	0.06

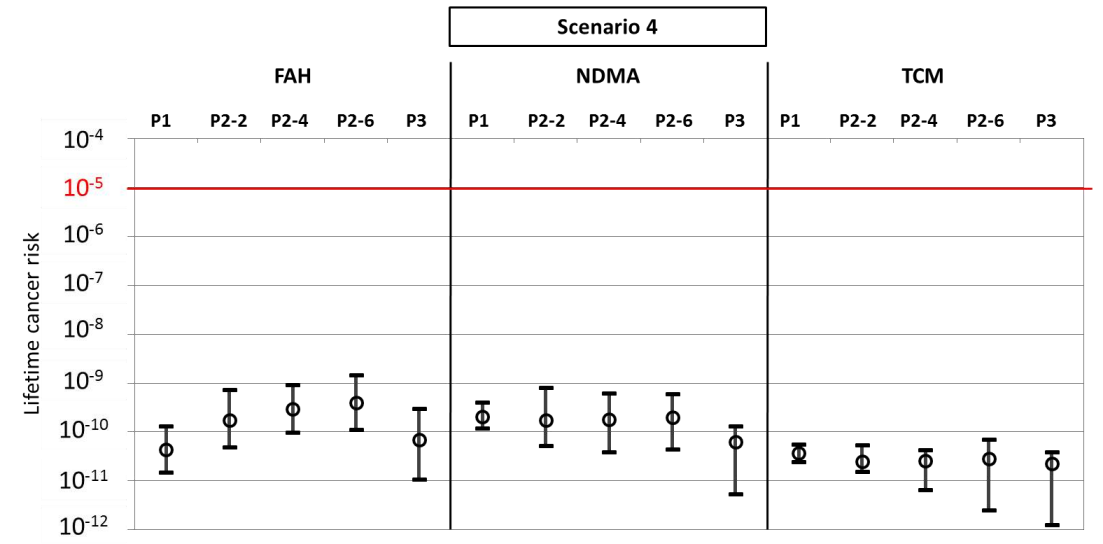
6.3.2.2 Estimated lifetime cancer risk

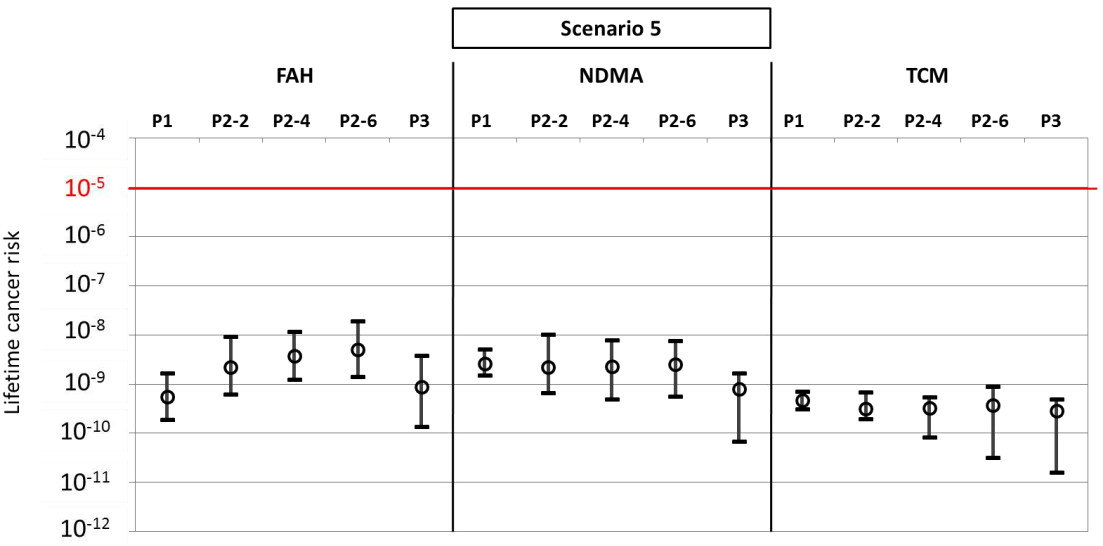
Lifetime cancer risk was calculated based on the estimated DBPs concentration in recycled water and exposure scenario (see Table S4, S5 and S6 in the supplementary material).

Figure 6.4 shows DBPs lifetime cancer risk for each scenario.




Scenario 3
FAH
NDMA
TCM
P1
P2-2
P2-4
P2-6
P3
Lifetime cancer risk
10-4
10-5
10-6
10-7
10-8
10-9
10-10
10-11
10-12


Scenario 4
FAH
NDMA
TCM
P1
P2-2
P2-4
P2-6
P3
Lifetime cancer risk
10-4
10-5
10-6
10-7
10-8
10-9
10-10
10-11
10-12


Scenario 5
FAH
NDMA
TCM
P1
P2-2
P2-4
P2-6
P3
Lifetime cancer risk
10-4
10-5
10-6
10-7
10-8
10-9
10-10
10-11
10-12

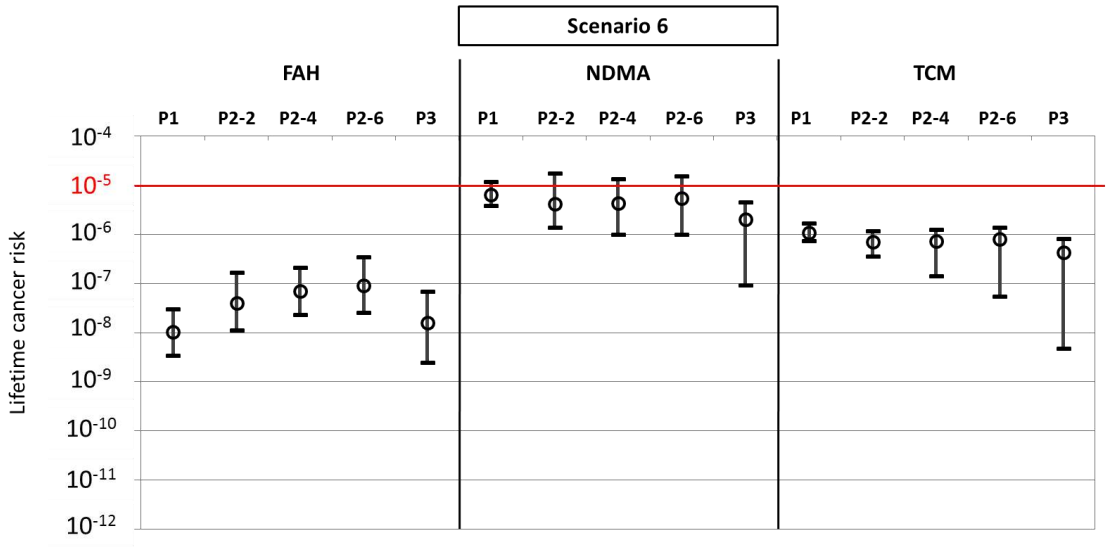


Figure 6.4 Lifetime cancer risk assessment for each exposure scenario

In scenario 1 to 5, lifetime cancer risk of 10^{-6} to 10^{-11} was obtained, and it was much smaller than 10^{-5}; WHO guideline values for THMs was associated with an excess cancer risk of 10^{-5} (WHO, 1993). Accordingly, lifetime cancer risk caused by DBPs in reclaimed water not seem to be a problem in scenario 1 to 5. Most of all, TCM has been considered that it has 'No significant risk level (NSRL)'. According to a report, NSRL of TCM is 20 μg/day via oral exposure and 40 μg/day via inhalation exposure (Office of Environmental Health Hazard Assessment [OEHHA], 2011). The concentration of TCM estimated in 6.3.2.1 was much lower than NSRL, and therefore it was expected that lifetime cancer risk caused by TCM is not be a problem in all scenario.

In scenario 6, FAH cancer risk was lower than 10^{-5}, while 10^{-5} to 10^{-7} of NDMA cancer risk was obtained. NDMA cancer risk in recycled water produced by P1 and P2 exceeded 10^{-5}, but it was possible to decrease NDMA cancer risk to below than 10^{-5}, by adding BAC treatment (P3). Therefore, O_3&CMF process with BAC was recommended to reduce NDMA cancer risk in scenario 6. In addition, FAH cancer risk increased with increasing ozone dosage, whereas there were no significant increases in NDMA cancer risk. On the contrary, a tendency that the 5th percentile value of NDMA cancer risk decreased with increasing ozone dosage was observed. It was attributed to the decreases of formation potentials of NDMA during ozonation.

6.3.3 Discussion on trade-off relationship between virus infection risk and lifetime cancer risk

It was expected that virus infection risk decreased while DBPs cancer risk increased with increasing ozone dosage. However, there was no significant difference between virus infection risk in reclaimed water produced by P2-2, P2-4 and P2-6. As described in Figure 6.4, moreover, lifetime cancer risk caused by FAH increased by about 10^{-1} with increasing ozone dosage, but it was lower than 10^{-5} of acceptable risk in all exposure scenario. In addition, there are no significant increases in lifetime cancer risk caused by NDMA or TCM with increasing ozone dosage.

Compared to reclaimed water produced by P1, virus infection risk decreased by 10^{-4} to 10^{-6} by incorporating ozonation although lifetime cancer risk caused by FAH increased slightly. The decreases of virus infection risk by incorporating ozonation was larger than the increases of lifetime cancer risk. Accordingly, it was possible to extend the uses of reclaimed water by incorporating ozonation regardless of the condition of ozone dosage.

6.3.4 Discussion on the uses of reclaimed water based on risk assessment

The uses of reclaimed water were evaluated based on risk assessment of virus infection and lifetime cancer risk. The result was summarized in Table 6.18.

As shown in Table 6.18, the reclaimed water produced by P1 was not suitable as the uses for scenario 1 and 6 from an aspect of virus infection risk and lifetime cancer risk, respectively, while it can be used for scenario 2 to 5. In case of the reclaimed water produced by P2, it can be used for scenario 1 to 5 regardless of ozone dosage tested in this study. However, it was unable to be used for scenario 6 because lifetime cancer risk exceeded acceptable risk. For using reclaimed water as the uses of scenario 6, it was necessary to be applied P3 which contains BAC treatment to reduce lifetime cancer risk.

Table 6.20 Evaluation of reclaimed water uses based on risk assessment

Scenario	Purpose	PACl+CMF (P1)			O_3+PACl+CMF (P2)									O_3+BAC+PACl+CMF (P3)		
					2 mg-O_3/L			4 mg-O_3/L			6 mg-O_3/L			6 mg-O_3/L		
		Virus	DBPs	**Total**	Virus	DBPs	**Total**	Virus	DBPs	**Total**	Virus	DBPs	**Total**	Virus	DBPs	**Total**
Scenario 1	Recreational impoundment	X	O	**X**	O	O	**O**	O	O	**O**	O	O	**O**	O	O	**O**
Scenario 2	Municipal irrigation	O	O	**O**	O	O	**O**	O	O	**O**	O	O	**O**	O	O	**O**
Scenario 3	Garden irrigation	O	O	**O**	O	O	**O**	O	O	**O**	O	O	**O**	O	O	**O**
Scenario 4	Toilet flushing	O	O	**O**	O	O	**O**	O	O	**O**	O	O	**O**	O	O	**O**
Scenario 5	Crop irrigation	O	O	**O**	O	O	**O**	O	O	**O**	O	O	**O**	O	O	**O**
Scenario 6	Unintended IPR	O	X	**X**	O	X	**X**	O	X	**X**	O	X	**X**	O	O	**O**

Virus infection risk : O < 10^{-6} $DALY_{pppy}$, X > 10^{-6} $DALY_{pppy}$
Lifetime cancer risk : O < 10^{-5}, X > 10^{-5} (O was determined only if lifetime cancer risk < 10^{-5} in all the examined three types of DBPs)
Virus infection risk in reclaimed water produced by P3 was estimated at same with that produced by P2

6.4 Conclusions

In this chapter, the assessment of both virus infection risk and lifetime cancer risk was conducted depending on the uses of reclaimed water. In addition, the applicability of reclaimed water for several uses was evaluated based on risk assessment.

The conclusion can be drawn as follows:

1. Virus infection risk from using recycled water produced by P1 met acceptable risk (10^{-6} DALYpppy) in scenario 2 to 6, but the 95th percentile virus infection risk was higher than the acceptable risk in scenario 1. It indicated that P1 was insufficient as treatment process when the uses of reclaimed water were recreational impoundment (scenario 1). However, infection risk due to exposure to viruses in recycled water produced by P2 met acceptable risk in all exposure scenarios.

2. In scenario 1 to 5, lifetime cancer risk of 10^{-6} to 10^{-11} was obtained, and it was much smaller than 10^{-5} of acceptable risk. Therefore, lifetime cancer risk caused by DBPs in reclaimed water not seem to be a problem in scenario 1 to 5. In scenario 6, however, NDMA cancer risk was higher than 10^{-5}. This NDMA cancer risk could decrease to below than 10^{-5} by adding BAC treatment. Therefore, O_3&CMF process with BAC was recommended to reduce NDMA cancer risk.

3. There was no significant difference between virus infection risk in recycled water produced by P2-2, P2-4 and P2-6. Lifetime cancer risk caused by FAH increased by about 10^{-1} with increasing ozone dosage, while there are no significant increases in lifetime cancer risk caused by NDMA or TCM. Compared to reclaimed water produced by P1, the decreases of virus infection risk by incorporating ozonation was larger than the increases of lifetime cancer risk. Accordingly, it was possible to extend the uses of reclaimed water by incorporating ozonation regardless of the condition of ozone dosage.

4. The reclaimed water produced by P1 can be used for scenario 2 to 5. In case of the reclaimed water produced by P2, it can be used for scenario 1 to 5 regardless of ozone dosage tested in this study. However, it was unable to be used for scenario 6 because lifetime cancer risk exceeded acceptable risk. For using reclaimed water as the uses of scenario 6, it was necessary to be applied P3

which contains BAC treatment to reduce lifetime cancer risk.

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Chapter Ⅶ

Investigation on occurrence and removal of indigenous virus and F-Specific RNA phages in ozonation and ceramic membrane filtration combination process based on RT-PCR assays

7.1 Introduction

Virus removal performance of ozonation and ceramic membrane filtration combination process (O_3&CMF process) was investigated in Chapter III and IV. These removal performances, however, were evaluated through experiments that MS2 were spiked artificially, thus there are insufficient information with regard to the removal of indigenous viruses in wastewater by O_3&CMF process. Although F-specific RNA phage (FPH) has been widely used as a surrogate of viruses from their morphological similarity with human enteric viruses, the association between the removal of human enteric viruses and that of MS2 spiked still remained unclear.

Moreover, there are several reports that FPH shows a different resistance in environment or during water treatment depending on their genotypes (Cole et al., 2003; Niapper et al., 2006; Boudaud et al., 2012; Haramoto et al., 2012; Hata et al., 2013; Yang et al., 2013). GI-FPH is most resistant to wastewater treatment, compared with other genotypes and human enteric viruses (Hata et al., 2013; Haramoto et al., 2015). It is expected that one of genotypes of indigenous FPH in wastewater has a potential as a conservative surrogate of viruses instead of spiked FPH artificially. However, the infectious FPH

genotypes was unknown since most of previous researches evaluated the resistance of FPH using reverse transcription - quantitative polymerase chain reaction (RT-qPCR) assay which provided information regarding only the presence or absence of specific DNA/RNA sequence regardless of whether viruses retain infectivity. The infectivity of viruses directly linked to public health risk is one of the most important issues in water reclamation, so rigorous evaluation of virus removal was required.

Although few research investigated genotyping of infectious FPH using a method combined plaque isolation with RT-qPCR (Haramoto et al., 2009, 2012, 2015; Gentry-Shields et al., 2015), these researches provided limited information regarding quantitative genotyping. A recent study reported that infectious FPH genotypes in surface water samples were successfully quantified using the application of RT-PCR based genotyping after FPH propagation in liquid medium (integrated culture [IC]-RT-PCR) (Hata et al., 2016). According to this study, furthermore, infectious FPH genotypes present at low concentration was effectively recovered without inactivation using a noncharged microfilter and $AlCl_3$ as coagulants. Therefore, it is expected that infectious FPH genotypes in samples which contain even low concentrations of FPH after ozonation or ceramic membrane filtration could be successfully quantified using IC-RT-PCR.

In this chapter, therefore, the removal of both indigenous virus and FPH in wastewater by O_3&CMF process were investigated. Furthermore, the removal of each genotype of infectious FPH was evaluated through quantitative genotyping using IC-RT-PCR assays. In addition, the obtained results were compared with that of MS2 spike test to investigate a difference between the removal performance of O_3&CMF process on indigenous viruses and MS2 artificially spiked.

7.2 Materials and Methods

7.2.1 Experimental methods and setup

All samples for the analysis of indigenous viruses were collected using experimental setup described in 4.2.3.1, 4.2.4.1 and 4.2.4.2. The experimental setup described in 4.2.3.1 was used for treating secondary effluent (SE), 4.2.4.1 and 4.2.4.2 were used for treating primary effluent (PE).

7.2.2 RT-qPCR

Indigenous viruses were analyzed by RT-qPCR assays. Target viruses were two genogroups of norovirus (GI and GII-NoV), aichi virus, pepper mild mottle virus (PMMoV) and three genogroups of FPH (GI, GII and GIII-FPH).

7.2.2.1 Sample concentration for RT-qPCR

The collected samples were concentrated by an adsorption-elution method, using an electronegative membrane (0.45μm pore size, HAWP09000, Millipore) (Katayama et al. 2002). This concentration method has been widely used for many studies related with virus in environmental or water treatment (Fong et al., 2005, 2010; Haramoto et al., 2005, 2013; Gersberg et al., 2006; Hansman et al., 2007; Gentry et al., 2009; Suzuki et al., 2011; Tian et al., 2011; Kitajima et al., 2011,2013; Hata et al., 2011, 2013). In brief, 2.5 mol/L of $MgCl_2$ was added to the sample to obtain a final concentration of 25 mmol/L. The sample, which contain $MgCl_2$, were passed through the electronegative membrane. After sample filtration, 200 mL of 0.5 mmol/L H2SO4 (pH 3.0) were also passed through the electronegative membrane in order to remove magnesium ions. Finally, viruses were eluted with 10 mL of 1.0 mmol/L NaOH (pH 10.8). The eluate was recovered in a tube containing 50 μL of 100 mmol/L H2SO4 (pH 1.0) and 100μL of 100 × Tris-EDTA buffer (pH 8.0) for neutralization. The eluates were concentrated once again using a centriprep YM-50 (Millipore) filter unit to obtain a final volume of 700 μL. The concentrated samples were stored at -80℃ until further analysis.

7.2.2.2 RT-qPCR assay

Viral RNA was extracted by using the QIAamp viral RNA mini kit (52904, QIAGEN). The extracted RNA was reverse transcribed with a High capacity cDNA Reverse transcription kit with RNase inhibitor (4374966, Applied Biosystems) using Thermal cycler dice gradient (TP600, Takara). Table 7.1 shows RT reaction thermal condition. Both extraction and reverse transcription was conducted according to the respective manufacturer's protocol.

The synthesized cDNA sample was mixed with TaqMan® gene expression master mix (4369016, Applied Biosystems), primers, and TaqMan probe. Table 7.1 and 7.2 shows the detail of the reaction mixture composition and thermal condition for TaqMan-based qPCR assay, respectively. The sequences of primers and probes were obtained from

previous studies, as shown in Table 7.4 to 7.10. TaqMan probe was labeled at 5' end with the FAM (6-Carboxyfluorescein) as the reporter dye, and TAMRA (6-carboxy-tetramethylrhodamine) or MGB-NFQ (Minor groove binder – Non fluorescent quencher) as the quencher dye was coupled in the 3' end. Thermal cycler dice real time system (TP800, Takara) was used for qPCR amplification. The genome copy numbers of each virus or phage were determined based on the standard curve prepared with 10-fold serial dilutions of plasmid DNA or oligo-DNA, containing each virus gene sequence to be amplified, at a concentration of 10^4 ~ 10^0 copies per reaction. The detection limit was about 100 copies/L (2 log (copies/L)) in this study.

Table 7.1 RT thermal condition

Step 1	Step 2	Step 3	Step 4
25℃ (10 min)	37℃ (120 min)	85℃ (5 min)	4℃ (∞)

Table 7.2 PCR reaction mixture composition

Component	Amount
2×Master Mix	12.5 μL
Sense primer	10 pmol
Antisense primer	10 pmol
TaqMan probe	2.5 pmol
cDNA template	5 μL
Nuclease free water	Adequate
Total	25 μL

Table 7.3 Real time PCR thermal condition

Stage 1 (1 cycle)		Stage 2 (50 cycle)	
Step 1	Step 2	Step 1	Step 2
Incubation	Enzyme Activation	Denaturation	Annealing and Extension
50℃ (2 min)	95℃ (10 min)	94℃ (15 s)	Appropriate temperatures depend on the used primer

Table 7.4 Sequences of primers and TaqMan probe for GI-NoV detection

Function	Sequence (5' → 3')	Length (nt)	Product size (bp)
Sense primer	CGYTGGATGCGNTTYCATGA	20	85
Antisense primer	CTTAGACGCCATCATCATTYAC	22	
TaqMan probe	FAM-AGATYGCGATCYCCTGTCCA-TAMRA	20	

Annealing temperature : 56℃

Reference : Kageyama et al., 2003.

Table 7.5 Sequences of primers and TaqMan probe for GII-NoV detection

Function	Sequence (5' → 3')	Length (nt)	Product size (bp)
Sense primer	CARGARBCNATGTTYAGRTGGATGAG	26	98
Antisense primer	TCGACGCCATCTTCATTCACA	21	
TaqMan probe	FAM-TGGGAGGGCGATCGCAATCT-TAMRA	20	

Annealing temperature : 56℃

Reference : Kageyama et al., 2003.

Table 7.6 Sequences of primers and TaqMan probe for AiV detection

Function	Sequence (5' → 3')	Length (nt)	Product size (bp)
Sense primer	GTCTCCACHGACACYAAYTGGAC	23	108-111
Antisense primer	GTTGTACATRGCAGCCCAGG	20	
TaqMan probe	FAM-TTYTCCTTYGTGCGTGC-MGB-NFQ	17	

Annealing temperature : 60℃

Reference : Kitajima et al., 2013.

Table 7.7 Sequences of primers and TaqMan probe for PMMoV detection

Function	Sequence (5' → 3')	Length (nt)	Product size (bp)
Sense primer	GAGTGGTTTGACCTTAACGTTGA	24	86
Antisense primer	TTGTCGGTTGCAATGCAAGT	20	
TaqMan probe	FAM-CCTACCGAAGCAAATG-MGB-NFQ	16	

Annealing temperature : 60℃

Reference : Zhang et al., 2006.

Table 7.8 Sequences of primers and TaqMan probe for GI-FPH detection

Function	Sequence (5' → 3')	Length (nt)	Product size (bp)
Sense primer	GTCCTGCTCRACTTCCTGT	19	82
Antisense primer	CGGCTACCTACAGCGATAG	20	
TaqMan probe	FAM-CAWGGTAGCGTCTCGCTAAAGACATTA -MGB-NFQ	27	

Annealing temperature : 58℃

Reference : Wolf et al., 2008.

Table 7.9 Sequences of primers and TaqMan probe for GII-FPH detection

Function	Sequence (5' → 3')	Length (nt)	Product size (bp)
Sense primer	TCTATGTATGGATCGCACTCG	22	111
Antisense primer	GTAGGCAAGTCCATCAAAGTC	21	
TaqMan probe	FAM-TGCTGTCCGATTTCACGTCTATCTTCA-MGB-NFQ	27	

Annealing temperature : 58℃

Reference : Wolf et al., 2008.

Table 7.10 Sequences of primers and TaqMan probe for GIII-FPH detection

Function	Sequence (5' → 3')	Length (nt)	Product size (bp)
Sense primer	GYGGTGCYACAACRACGAAT	20	77
Antisense primer	GWGGSGTACACKCTTGCG	18	
TaqMan probe	FAM-TACGGYCATCCGTCCTTCAAGTTTG-MGB-NFQ	25	

Annealing temperature : 58℃

Reference : Wolf et al., 2008.

7.2.3 IC-RT-PCR assay

In this study, infectious FPH was analyzed using both IC-RT-PCR and plaque assay. IC-RT-PCR method has been used as one of methods which can analyze the infectivity of viruses by conducting a cultivation step prior to PCR (Li et al., 2010; Gao et al., 2013; Vergara et al., 2015; Hata et al., 2016). The infectivity of individual FPH genotypes was analyzed qualitatively through sample concentration and IC-RT-PCR even if samples contain low concentration of FPH, and then it was quantified by the application of most probable number (MPN) method. Meanwhile, total infectious FPH regardless of genotypes was analyzed by plaque assay. Table 7.11 shows culture medium composition. Figure 7.1 shows the experiment flow for IC-RT-PCR. Samples of 10-fold serial dilutions (1000 mL to 1mL or 100 mL to 0.1mL), each dilution has 3 aliquots, were prepared for one sample analysis. Samples were diluted appropriately if high FPH concentration were expected.

Table 7.11 Culture medium composition

Component	Unit	Liquid medium	2×Liquid medium	Solid medium
Milli-Q	mL	500	500	500
Trypton	g	5	10	5
Glucose	g	0.5	1.0	0.5
NaCl	g	4	8	4
$CaCl_2 \cdot 2H_2O$ (0.3g/mL)	mL	0.5	1.0	0.5
$MgSO_4 \cdot 7H_2O$ (0.15g/mL)	mL	0.5	1.0	0.5
Bacto Agar	g	-	-	5.5
Kanamycin (20g/L)	mL	0.5	1.0	0.5
Nalidixic Acid (100g/L)	mL	0.5	1.0	0.5

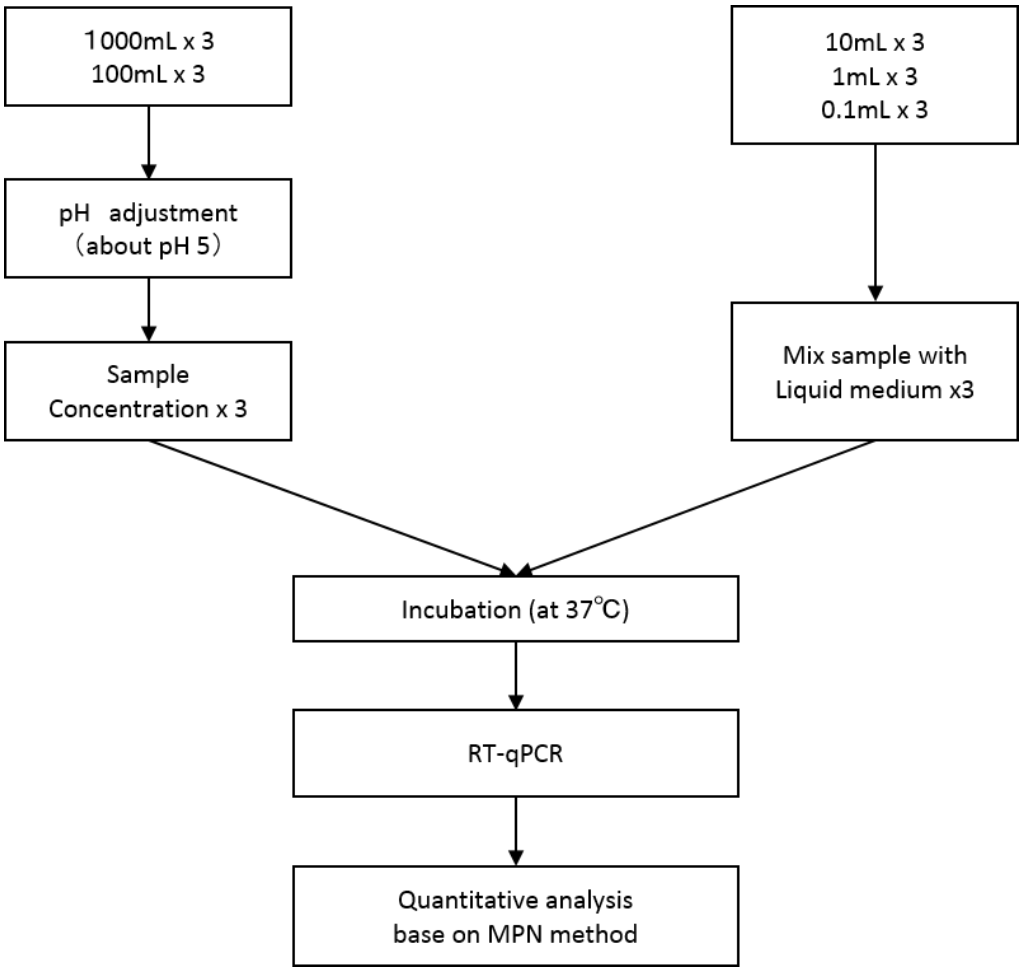


Figure 7.1 Experimental procedure of IC-RT-PCR

7.2.3.1 Sample concentration for IC-RT-PCR

Samples were concentrated if it was expected that samples have low FPH concentration, below than 1 PFU/mL. A coagulant and a host were added into the sample which have necessary to concentration procedure after the pH of sample was adjusted to pH 5.0 with hydrochloric acid. Aluminum chloride solution of 250 mM ($AlCl_3$) was used as coagulant. *Salmonella enterica serovar typhimurium* WG49 (WG49), propagated for 6 ~ 8 hours before experiment, was used as bacterial host. The efficiency of FPH concentration would be improved by adding both coagulant and host into sample before filtration, and also adjusting the pH of sample. Both coagulant and host were added into samples to be diluted them 200 times. Samples of 1000 mL or 100 mL were filtered through HV membrane (0.45 μm pore size, HVLP04700; HVLP09050, Millipore). After filtration, the filter was put in a petri dish added liquid agar in advance. The petri dish was

incubated for 18 ~ 24 hours at 37℃. Samples were mixed with the same volume of liquid medium directly if those were no need to concentration procedure, in case of 10, 1, 0.1 mL. The mixtures were then incubated for 18 ~ 24 hours at 37℃. Samples which finished incubation was stored at 4℃ until further analysis.

7.2.3.2 IC-RT-PCR assays

The infectious FPH genotyping was conducted using one-step RT-PCR kit. QuantiTect Probe RT-PCR kit (204443, Qiagen) was used for PCR amplification. These one-step RT-PCR kit allows both RT and PCR to take place in a single tube. Therefore, it was possible to minimize contamination, and also reduce experimental procedure.

The cultured sample of 2 µL was added to 96-well PCR plate. The cultured sample in the plate was subjected to RNA extraction by heating at 95℃ for 5 min using Thermal cycler dice gradient (TP600, Takara) in this analysis. Reaction mixtures for IC-RT-PCR was added to the PCR plate after RNA extraction. Table 7.12 shows the detail of reaction mixtures composition for IC-RT-PCR. The sequences of primers and probes were referred to Table 7.4 ~ 7.11. Thermal cycler dice real time system (TP800, Takara) was used for PCR amplification. Table 7.13 shows thermal condition for IC-RT-PCR. The standard curve was unnecessary because this PCR amplification had an objective for qualitative analysis. FPH concentration was quantified base on MPN method after verification whether each FPH genotype in specimens was positive or not.

Table 7.12 Composition of reaction mixture for IC-RT-PCR

Component	Amount
Master Mix	10 µL
Sense primer	8 pmol
Antisense primer	8 pmol
TaqMan probe	4 pmol
RT Mix	0.2 µL
RNA template	2 µL
Nuclease free water	Adequate
Total	20 µL

Table 7.13 Thermal condition for IC-RT-PCR

Stage 1 (1 cycle)		Stage 2 (40 cycle)	
Step 1	Step 2	Step 1	Step 2
Incubation	Enzyme Activation	Denaturation	Annealing and Extension
50℃ (30 min)	95℃ (15 min)	94℃ (15 s)	58℃ (60 s)

7.2.4 Plaque assays

7.2.4.1 Indigenous FPH

Infectious FPH was quantified as described in ISO standard 10705-1 using host strain WG49. Culture medium composition was shown in Table 7.11.

7.2.4.2 Bacteriophage MS2

Bacteriophage MS2 was selected as model virus. MS2 spike test and MS2 analysis was conducted in accordance with methods described in 3.2.1.

7.3 Results and discussion

7.3.1 Occurrence and removal of indigenous viruses and FPH in O_3&CMF process for treating secondary effluent

7.3.1.1 Occurrence and removal of indigenous viruses and FPH in O_3&CMF process for treating secondary effluent

Figure 7.2 shows occurrence of GI-NoV, GII-NoV, AiV and PMMoV in O_3&CMF process for treating SE. Values on each figure represent O3 dosage (mg-O3/L). PACl dosage was commonly 25 mg-PACl/L.

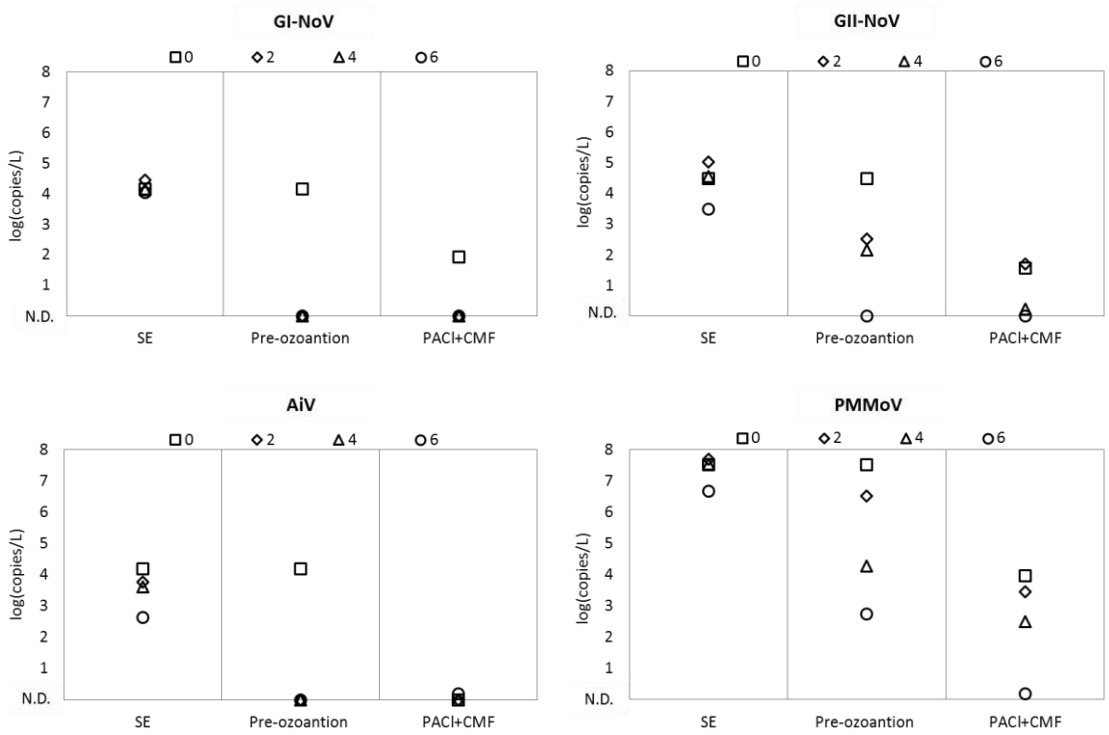


Figure 7.2 Concentrations of GI-NoV, GII-NoV, AiV and PMMoV in O_3&CMF process for treating SE (Values on each figure represent O_3 dosage (mg-O_3/L). PACl dosage was commonly 25 mg-PACl/L. N.D. means Not detected.)

Mean concentrations of GI-NoV, GII-NoV, AiV and PMMoV in SE were 1.5 x 10^4, 4.3 x 10^4, 5.3 x 10^3 and 2.2 x 10^7 copies/L, respectively. These viruses were hardly removed by only ceramic membrane filtration, because they have smaller sizes than pore size. However, virus concentrations of GI-NoV, GII-NoV, AiV and PMMoV in CM permeates decreased to 8.4x10^1, 3.6x10^1, N.D., 9.3x10^3 copies/L, respectively, by the addition of PACl (25 mg/L). These viruses were much effectively removed by incorporating ozonation as the pretreatment of CM. GI-NoV and AiV was not detected under all of ozone dosage conditions, and the concentration of GII-NoV was below than 10^2 copies/L in pre-ozonated water. In case of PMMoV, which has the highest concentration in SE, was detected at the concentration of 3.3 x 10^6 1.9 x 10^4 and 5.6 x 10^2 copies/L in 2, 4 and 6 mg-O_3/L of pre-ozonated water, respectively.

In O_3&CMF process, consequently, GI-NoV and AiV in CM permeates was not detected under all of tested conditions, except for the case that AiV was detected at amounts near the detection limit (2 copies/L) under the condition of 6/25. GII-NoV and PMMoV were below the detection limit and 2.0 x 10^0 copies/L, respectively, under the ozone dosage of 6mg/L.

Figure 7.3 shows occurrence of GI, GII and GIII-FPH in O_3&CMF process for treating SE.

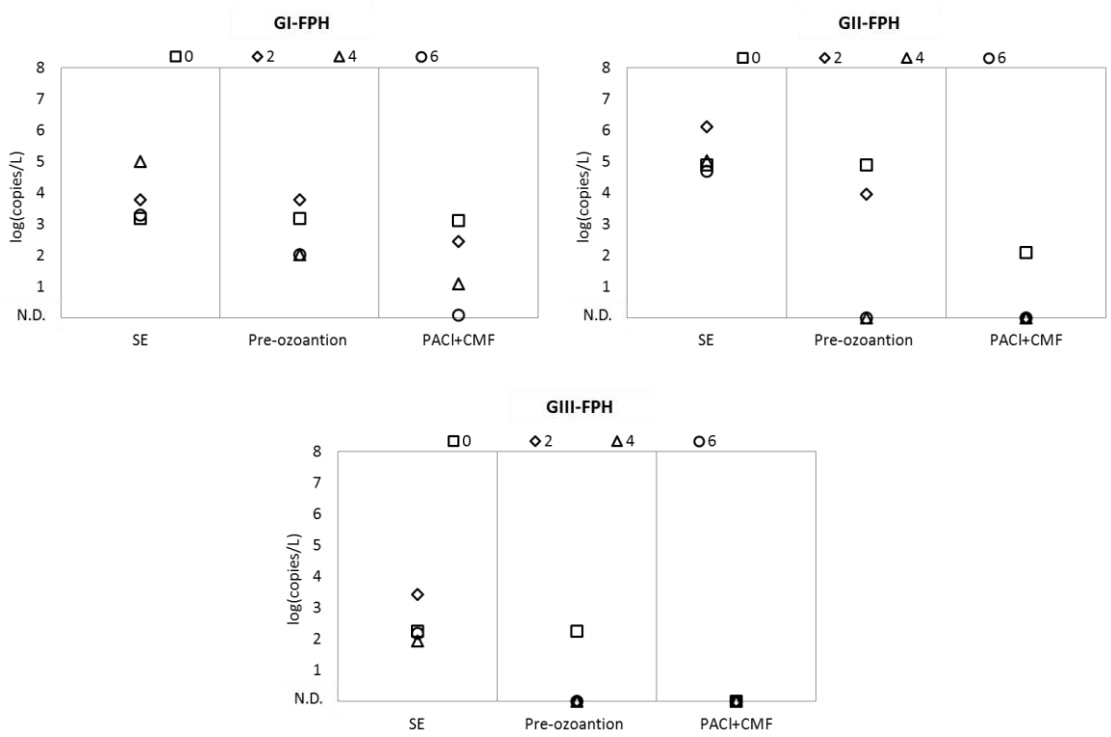


Figure 7.3 Concentrations of GI-FPH, GII-FPH and GIII-FPH in O_3&CMF process for treating SE (Values on each figure represent O_3 dosage (mg-O_3/L). PACl dosage was commonly 25 mg-PACl/L. N.D. means Not detected.)

Mean concentrations of GI, GII and GIII-FPH in SE were 2.6 x 10^4, 5.7 x 10^5 and 8.0 x 10^2 copies/L, respectively. Although both GI and GII-FPH concentration was slightly decreased after CMF, it was revealed that only CMF is difficult to remove viruses effectively. However, GI and GII-FPH concentration in CM permeates decreased to 1.3 x 10^3, 1.2 x 10^2 copies/L through PACl (25mg/L)+CMF. By incorporating ozonation with PACl+CMF, GII-FPH was not detected in CM permeates, and GI-FPH concentration decreased from 2.8 x 10^2 to 1.0 x 10^0 copies/L with increasing ozone dosage from 2 to 6 mg/L. GIII-FPH, which has relatively low concentrations in SE, was not detected in CM permeates in all tested condition. Comparing with PACl+CMF, FPH was more effectively removed in O_3&CMF process, similar to the result of human enteric viruses. Furthermore, GI-FPH showed the tendency that they are difficult to be removed by combination, compared to GII-FPH and other viruses. Although several researches has been reported that this tendency appeared in wastewater treatment process (Hata et al.,

2013; Haramoto et al., 2015), it is a first report that the similar tendency were observed in not only PACl+CMF but also ozonation. Meanwhile, it was expected that ozonation would be much more effective on virus inactivation rather than the degradation of viral DNA or RNA. The virus infectivity is one of the most important factors on the hygienic evaluation of reclaimed water. Thus, not only the removal of indigenous viruses but also the inactivation of FPH by O_3&CMF process was investigated through RT-PCR and IC-RT-PCR in following sections.

7.3.1.2 Removal of indigenous viruses and FPH and comparison with MS2

7.3.1.2.1 Pre-ozonation

Figure 7.4 shows indigenous virus removal during post-ozonation for treating SE. The experiment was triplicated. Mean removal rate of indigenous virus during pre-ozonation can confirm in the supplementary material (Figure S5). The horizontal axis and vertical axis represents O_3 consumption (mgO_3/mgC) and removal rate (log), respectively. N.D. represents that virus was not detected in ozonated water. The removal rate was calculated using detection limit of concentration if virus was not detected in ozonated water.

GI-NoV removal rate of 0.4 to 2.5 log was obtained under 0.10 to 0.48 mgO_3/mgC, and AiV removal rate was 0.1 to 1.6 log. Both GI-NoV and AiV was N.D. in ozonated water of 0.57 mgO_3/mgC. On the other hand, the removal rate of GII-NoV was 0.1 to 2.7 log under 0.10 to 0.57 mgO_3/mgC. GII-NoV showed most a large variability in removal rate compared with other viruses. The slope (increases of removal rate against ozone consumption) of trend line was 5.17, 0.88 and 3.79 $log/mgO_3/mgC$ for GI-NoV, GII-NoV and AiV, respectively, indicating that GII-NoV is difficult to be removed during ozonation. According to Shin et al. (2003), 4 log of NoV (and 5 log of poliovirus) reduction was obtained under 0.37 mg-O_3/L in buffered ozone demand free water for 5 min reaction. This reported log reduction was quite higher than our results. This difference seems to be caused by a particle shielding effect. As mentioned in 4.3.1.2, virus aggregated each other or associated with particles is difficult to be inactivated through disinfection (Ormeci and Linden, 2002; Templeton et al., 2005; Shin et al., 2008). In case of PMMoV, most abundant in SE among tested viruses, the highest removal rate of 3.0 log was observed under 0.48 mgO_3/mgC, while it was rarely removed until 0.19 mgO_3/mgC. Ozone dosage seems to be required over certain level in order to destroy viral capsid and degrade viral RNA. The trend line slope of PMMoV was 3.96 $log/mgO_3/mgC$.

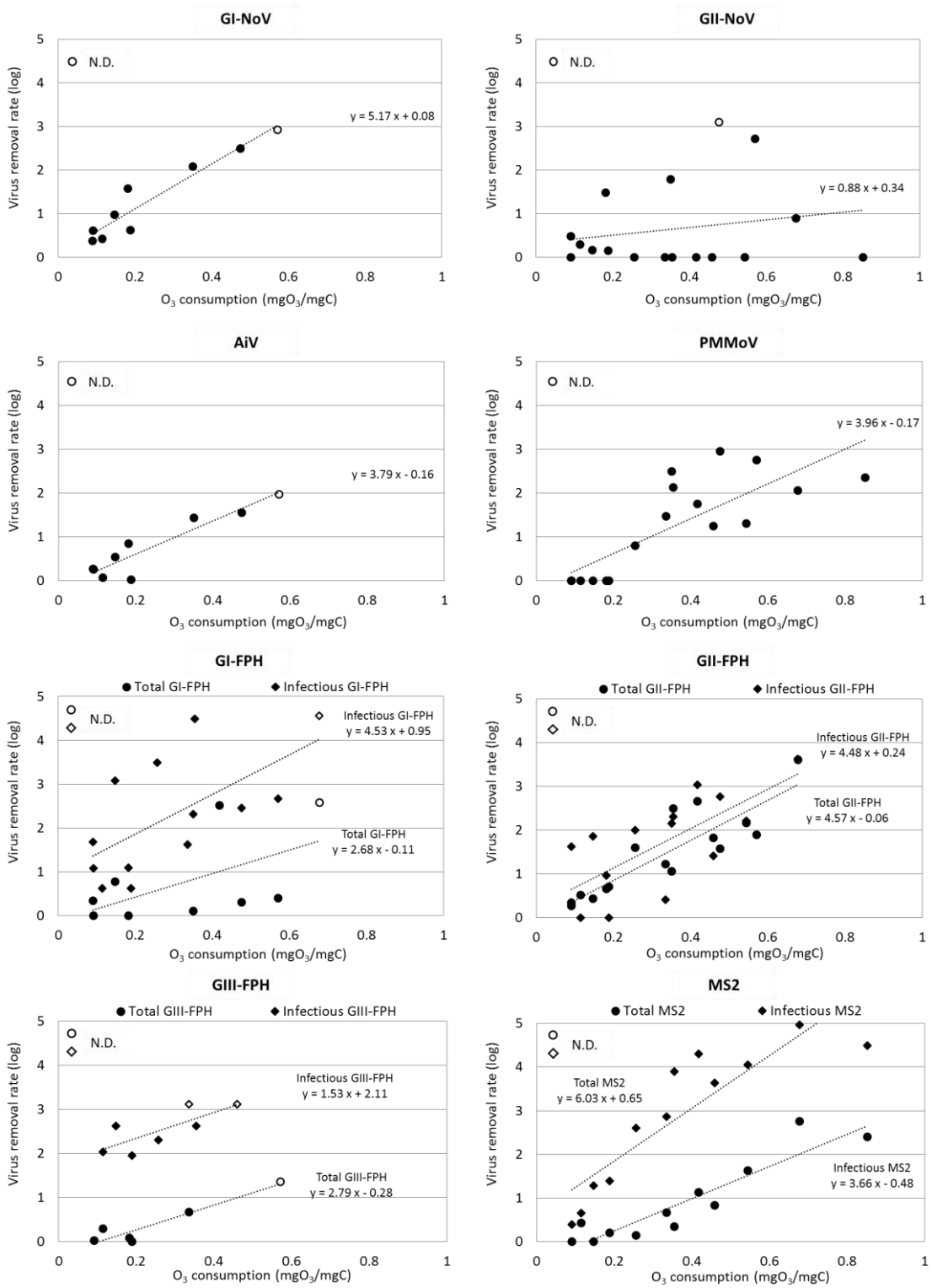


Figure 7.4 Indigenous virus and the spiked MS2 removal rates during pre-ozonation (A dotted line represents trend line of the removal rate against mgO_3/mgC. White circle or diamond indicates that virus was not detected in ozonated water and represents the highest calculated removal rate.)

In case of FPH removal rate, the removal rate calculated from FPH concentration quantified by RT-qPCR and by IC-RT-PCR was defined as total and infectious FPH removal rate, respectively. The removal rate of indigenous FPH was 0.3 to 2.7 log under 0.10 to 0.48 mgO_3/mgC, regardless of the retention of their infectivity. Infectious FPH showed over 1 log inactivation at 0.10 mgO_3/mgC, and 2 to 3 log of inactivation was observed at 0.48 mgO_3/mgC. The removal rate of infectious FPH was approximately 1 to 2 log higher than that of total FPH, indicating that ozonation is more effective on virus inactivation than the degradation of viral RNA. On the basis of these results, it can be assumed that 1 to 3 log inactivation of human enteric viruses were able to be achieved under 0.10 to 0.48 mgO_3/mgC. A further study on the inactivation of human enteric viruses is required to prove the assumption.

With respect to each genotype of FPH, total removal rate of GII-FPH (0.4 to 2.7 log) was generally higher than that of GI-FPH (0.2 to 2.5 log). Furthermore, the trend line slope of total GII-FPH removal (4.57 log/mgO_3/mgC) was higher than that of total GI-FPH removal (2.68 log/mgO_3/mgC). Interestingly, however, there was no significant difference in log inactivation between infectious removal rate of GI-FPH and GII-FPH (the removal rate was both 1 to 4 log, and the slope was 4.53 and 4.48 log/mgO_3/mgC for infectious GI and GII-FPH, respectively). Moreover, it was found that GII-FPH shows most small difference between the removal rate of total and infectious removal rate (the slope of total and infectious GII-FPH was 4.57 and 4.48 log/mgO_3/mgC, respectively), compared to the other FPH genotypes. This can be explained by the ratio of FPH which retain the infectivity in source water. The infectivity index, defined as the difference between the concentration of infectious FPH and total FPH (log (MPN/copies)), of each FPH genotypes in SE showed that GII-FPH has a lowest value among three FPH genotypes (see Figure S6 (a) in the supplementary material). It indicated that the ratio of GII-FPH which retain infectivity was relatively low, although the concentration of total GII-FPH, quantified by RT-qPCR assays, was most high in SE. Therefore, there is a possibility that a large amount of GII-FPH is present as the form of liberated viral RNA in SE, and it could be lead to a lot of viral RNA degradation by ozonation. In case of GIII-FPH, total GIII-FPH was N.D. in ozonated water of 0.57 mgO_3/mgC, and infectious GIII-FPH was readily inactivated (2 log of inactivation under 0.1 mgO_3/mgC). As mentioned above, the tendency that GIII-FPH is less persistent during ozonation than GI and GII-FPH was observed.

The removal rate of MS2 and GI-FPH was relatively similar at low O_3 consumption (0.1 mgO_3/mgC), but the removal rate of MS2 overtook that of GI-FPH with increasing ozone dosage. The trend line slope of MS2 was higher than that of GI-FPH (2.68 and 4.53

log/mgO_3/mgC for total and infectious GI-FPH; 3.66 and 6.03 log/mgO_3/mgC for total and infectious MS2). Furthermore, GI-FPH showed a larger variability in the removal rate than MS2. Even though MS2 is a representative strain of GI-FPH, there are apparent differences between the removal behaviors of them. It might be attributed to the difference between the concentrations in source water. GI-FPH concentrations was daily (and seasonally) variable (10^2 to 10^5 copies/L), in common with the other indigenous viruses, whereas the concentration of spiked MS2 was fixed at 10^6 to 10^7 PFU/ml. In addition, GI-FPH might be present as the form associated with particles, compared to MS2 purified prior to spike test. It could be the other reason. Consequently, GI-FPH shows much similar removal behaviors to human enteric virus such as GII-NoV, which indicates the potential as alternative surrogates.

In comparison with above human enteric viruses, GII-FPH showed higher removal rate, and similar or slight lower removal rates were obtained from GI-FPH.

7.3.1.2.2 O_3+PACl+CMF

In O_3+PACl+CMF for treating SE, the concentration of indigenous viruses was detected at the level of detection limit or N.D. after pre-ozonation. Thus, it was difficult to evaluate only removal rate by PACl+CMF. For this reason, the removal rate of indigenous virus by O_3+PACl+CMF was described in Figure 7.5. The experiment at each experimental condition was conducted once on different dates. The horizontal axis and vertical axis represents O_3 dosage (mg-O_3/L) and removal rate (log), respectively. The removal rate was calculated using detection limit of concentration if virus was not detected in CM permeate. Total and infectious FPH removal rate indicate the removal rate calculated from the concentration quantified by RT-qPCR and by IC-RT-PCR, respectively.

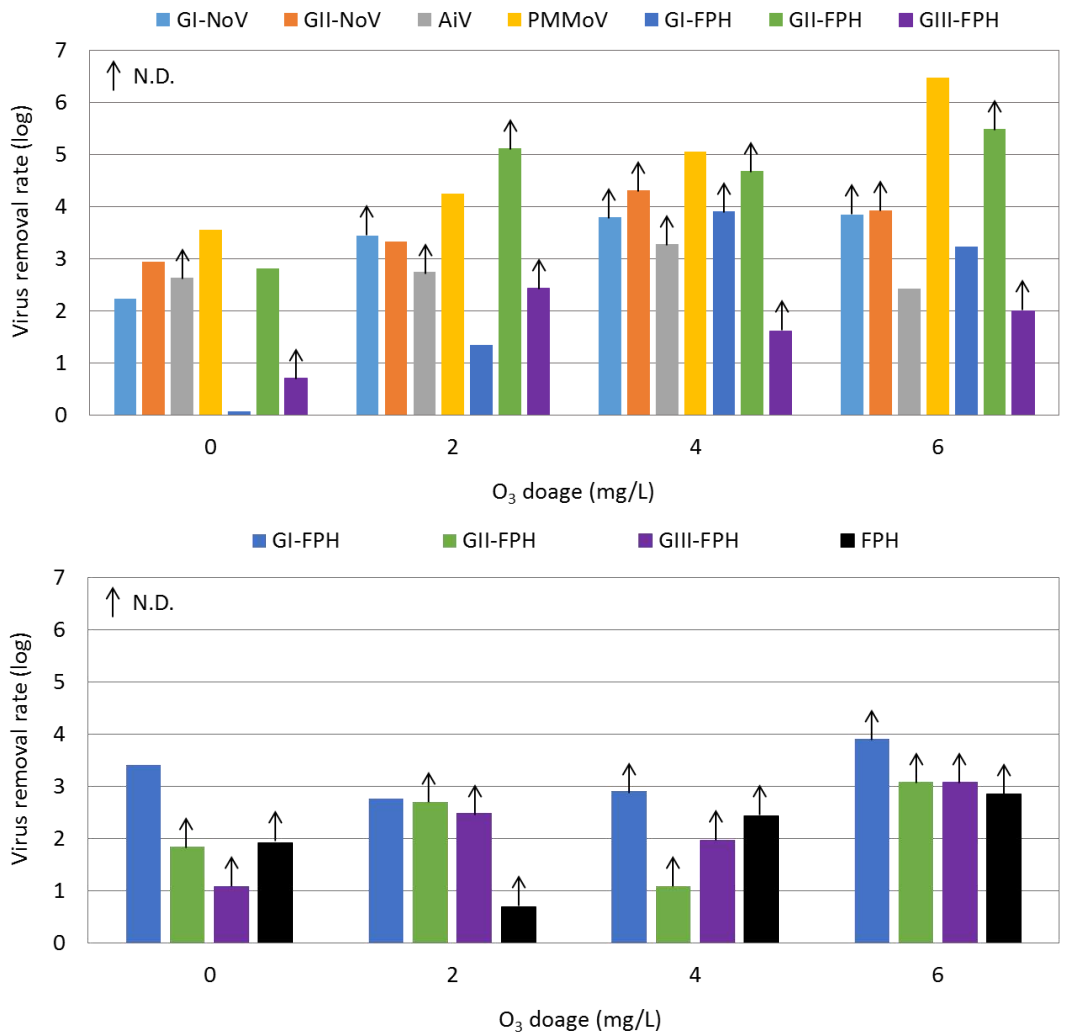


Figure 7.5 The removal rates of (a) total indigenous viruses and (b) infectious FPH by O_3+PACl+CMF (PACl dosage was commonly 25 mg-PACl/L. The allow above each bar graph represent that viruses was not detected in the CM permeate)

About 2.2 and 2.9 log of GI-NoV and GII-NoV removal rate, respectively, was observed by PACl+CMF. However, the concentration of them was N.D. in CM permeate by incorporating pre-ozonation, and as a result higher than 3 log of removal rate was obtained. AiV was N.D. in CM permeate except for the condition of 6 mg-O_3/L. AiV was detected at the level of detection limit (about 1 copies/L) under the condition of 6 mg-O_3/L, and 2.7 log of AiV removal rate was obtained. PMMoV was detected in all CM permeate, and 3.6 log of removal rate was obtained by PACl+CMF, and it increased to 6.6 log by incorporating pre-ozonation of 6mg/L.

In case of FPH removal, 3.4 log of infectious GI-FPH was inactivated by PACl+CMF

while total GI-FPH was rarely removed. It indicated that GI-FPH was inactivated during PACl+CMF. The inactivation by PACl might be one of that reason. It has been well reported that FPH was inactivated by the addition of coagulants such as PACl (Matsui et al., 2003; Shirasaki et al., 2009, 2016; Guo and Hu, 2011; Matsushita et al., 2011, Kreißel et al., 2014). By incorporating pre-ozonation, 3.2 and > 4 log of total and infectious GI-FPH removal rate was observed under the condition of 6 mg-O_3/L, respectively. On the other hand, total GII-FPH removal rate was 2.8 log by PACl+CMF, and it increased to > 4.5 log by incorporating pre-ozonation. Infectious GII-FPH was N.D. in all CM permeate. The removal rate of both total and infectious GI-FPH was much lower than that of GII-FPH. As similar with the result of pre-ozonation, it was found that GI-FPH is difficult to be removed, compared to not only the other FPH but also human enteric viruses. Meanwhile, both total and infectious GIII-FPH was N.D. in CM permeate under all tested condition, indicating that GIII-FPH is readily removed by O_3+PACl+CMF.

Unfortunately, MS2 spike test was not conducted by continuous O_3+PACl+CMF in Chapter IV, so it was difficult to compare the removal of GI-FPH and MS2 by O_3+PACl+CMF. However, it was found that the removal of infectious GI-FPH by PACl+CMF (3.2 log) was much lower than that of MS2 (8.3 log). This result indicated that indigenous GI-FPH is much difficult to be removed by PACl+CMF than spiked MS2. There was a possibility to be overestimated virus removal by PACl+CMF in Chapter IV. For this reason, the evaluation of virus removal performance was reconsidered in 7.3.3 using GI-FPH, which showed a potential as most conservative surrogate virus in this chapter.

7.3.2 Occurrence and removal of indigenous viruses and FPH in O_3&CMF process for treating primary effluent

7.3.2.1 Occurrence of indigenous viruses and FPH in O_3&CMF process

Figure 7.6 shows occurrence of GI-NoV, GII-NoV, AiV and PMMoV in O_3&CMF process for treating PE. Post-ozonation was conducted using PACl(50mg/L)+CMF permeate as a source water.

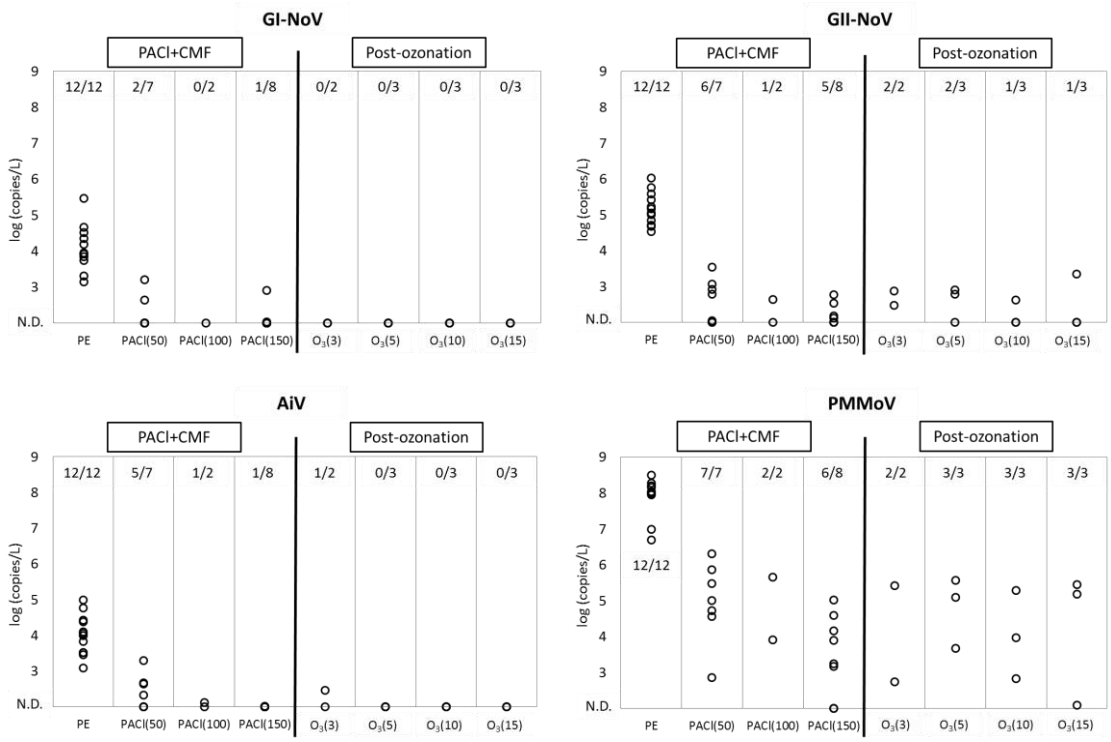


Figure 7.6 Concentrations of GI-NoV, GII-NoV, AiV and PMMoV in O_3&CMF process for treating PE (Numbers in parentheses of the horizontal axis represent PACl or O_3 dosage. Numbers above each graph item indicate the number of positive samples/total samples. N.D. means Not detected.)

Mean concentrations GI-NoV, GII-NoV, AiV and PMMoV in PE were 2.1 x 10^4, 2.8 x 10^5, 3.7 x 10^4 and 1.3 x 10^8 copies/L, respectively. GI-NoV and AiV concentrations largely decreased by PACl+CMF, and as a result their concentrations were below 10^3 copies/L even under 50 mg/L of PACl dosage. GI-NoV and AiV were not detected in post-ozonated water, except that AiV was once detected at detection limit level under 3mg-O_3/L. GII-NoV showed similar concentrations in CM permeate with GI-NoV or AiV, but the positive ratio was much higher (71% for GII-NoV; 18% for GI-NoV; 41% for AiV). Moreover, GII-NoV was still detected at the concentration of 10^3 copies/L, after post-ozonation, although the positive ratio of GII-NoV slightly decreased. These results were indicated that the reclaimed water produced from PE can contain larger amounts of GII-NoV than GI-NoV or AiV. Meanwhile, PMMoV, which had a highest concentration in PE among tested viruses, was detected at 10^4 ~10^6 copies/L in CM permeate (50mg/L of PACl). Although PMMoV concentrations in CM permeate decreased with increasing PACl dosage, it was detected at relatively high concentrations of 10^3 ~ 10^5 copies/L under 150 mg-PACl/L. Moreover, PMMoV was detected at 10^3to10^5 copies/L in post-ozonated water

(100% of the positive ratio), indicating that their concentrations were not significantly changed after post-ozonation. It means that the viral RNA of PMMoV is difficult to be degraded by ozonation, compared to the other viruses. As mentioned above, the morphological difference might be those reasons.

Figure 7.7 shows occurrence of GI-FPH, GII-FPH and GIII-FPH in O_3&CMF process for treating PE. Post-ozonation was conducted using PACl(50)+CM permeate as a source water.

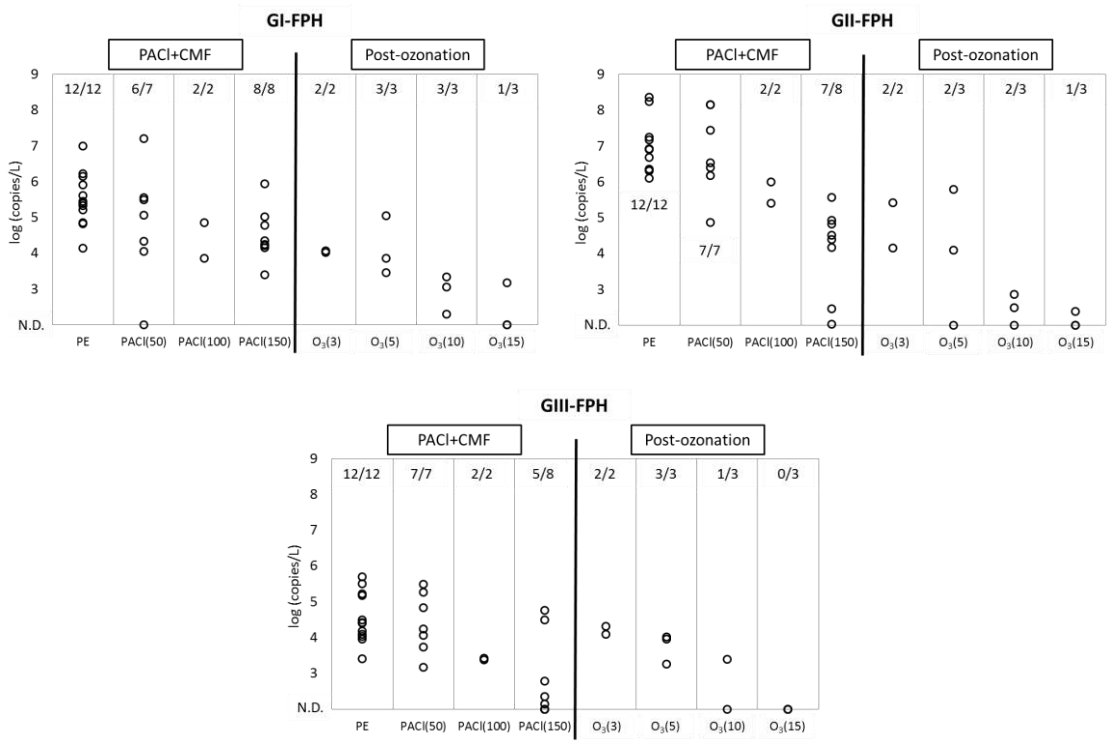


Figure 7.7 Concentrations of GI-FPH, GII-FPH and GIII-FPH in O_3&CMF process for treating PE (Numbers in parentheses of the horizontal axis represent PACl or O_3 dosage. Numbers above each graph item indicate the number of positive samples/total samples. N.D. means Not detected.)

Mean concentrations of GI, GII and GIII-FPH in PE were 1.2 x 10^6, 8.4 x 10^7 and 9.9 x 10^4 copies/L, respectively. FPH concentrations were not significantly changed after PACl(50)+CMF. GI, GII and GIII-FPH were detected in range of 10^3 ~ 10^6, 10^2 ~ 10^5 and 10^2 ~ 10^5 copies/L, respectively, in CM permeate with PACl(150). As opposed to the above results of human enteric viruses, interestingly, FPH showed comparatively small reductions in concentrations by PACl+CMF. In addition, the positive ratio of them was

higher than that of human enteric viruses (94%, 94% and 82% for GI, GII and GIII-FPH, respectively) in CM permeate. These results suggested that more PACl dosage is required to obtain FPH removal rate similar with human enteric virus by PACl+CMF. During post-ozonation, FPH concentrations gradually decreased with increasing ozone dosage, and as a result all of three FPH genotypes were detected at a maximum level of 10^3 copies/L under 10 mg-O_3/L. FPH showed apparent tendency for the decreases of concentration during post-ozonation, compared to human enteric viruses. This tendency seems to be due to the relatively higher FPH concentrations in CM permeate (10^2 to 10^5 times higher than human enteric viruses), which resulted from the small reduction by PACl+CMF. Among three FPH genotypes, GI-FPH showed most high concentrations and the positive ratio in reclaimed water, indicating a high persistence during O_3&CMF process, the same result as above.

In addition, it was found that there is the difference between removal characteristics of human enteric viruses and FPH by PACl+CM and post-ozonation. In the next section, the comparison on the removal of human enteric viruses and FPH would be discussed in detail.

7.3.2.2 Removal of indigenous viruses and FPH and comparison with MS2

7.3.2.2.1 PACl+CMF

Figure 7.8 shows mean removal rates of indigenous viruses by PACl+CMF for treating PE. The experiment at 50 and 100 mg/L of PACl dosage was duplicated and the experiment at 150 mg/L of PACl dosage was octuplicated. The removal rate was calculated using detection limit of concentration if virus was not detected in CM permeate. Total and infectious FPH removal rate were indicate the removal rate calculated from the concentration quantified by RT-qPCR and by IC-RT-PCR, respectively.

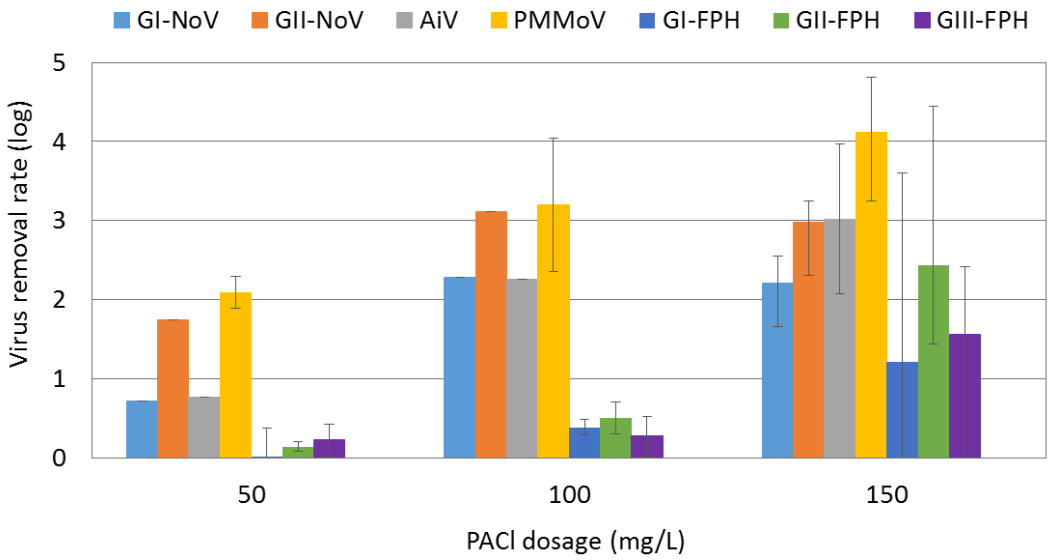


Figure 7.8 Mean removal rates of total indigenous viruses by PACl+CMF for treating PE (The value indicates mean removal rate by PACl+CMF, and error bar represents the range)

A virus removal rate ranged from 0 to 2 log was obtained by PACl(50mg/L)+CMF. Virus removal rate by PACl+CMF increased with increasing PACl dosage, and it was obtained ranging of 1 to 4 log by PACl(150mg/L)+CMF. In terms of each viruses, the removal rate of GI-NoV, GII-NoV, AiV and PMMoV was 0.7 to 2, 1.5 to 3, 0.7 to 3 and 2 to 4 log, respectively. According to several researches, 1 to 3 log of NoV, 1 log of AiV and PMMoV removal rate was observed during wastewater treatment (conventional activated sludge) (Hata et al., 2013; Kitajima et al., 2014; Haramoto et al., 2015). Much higher virus removal rate could be obtained by PACl(150mg/L)+CMF. Meanwhile, 0.2 to 2.4 log of FPH removal rate was observed, and it was much smaller removal rate compared to human enteric viruses. Especially, less than 0.5 log of FPH was removed by PACl(100mg/L)+CMF, while 2 to 3 log of human enteric viruses was removed. It indicated that abundant PACl dosage was required to remove FPH effectively, as opposed to human enteric viruses effectively removed by even relatively smaller PACl dosage. The result that viruses are difficult to be removed only by CMF has already shown (see Figure S2 in the supplementary material). Thus, it was considered that there is difference between the coagulation efficiency of human enteric viruses and FPH. The removal rate of human enteric viruses was 0.5 to 1.5 log higher than that of FPH by coagulation (see Figure S7 in the supplementary material). The coagulation of indigenous virus would be affected by the complicated reasons such as the properties of particles and viruses, and therefore a further study on this difference between the coagulation of human enteric

viruses and FPH was required. In terms of FPH genotypes, high removal rate observed in order of GII-FPH, GIII-FPH and GI-FPH. As similar with the result of ozonation, GI-FPH showed most low removal rate.

To further understand, the removal of each FPH genotype including their infectivity was investigated, and it compared to the result of MS2 spike test.

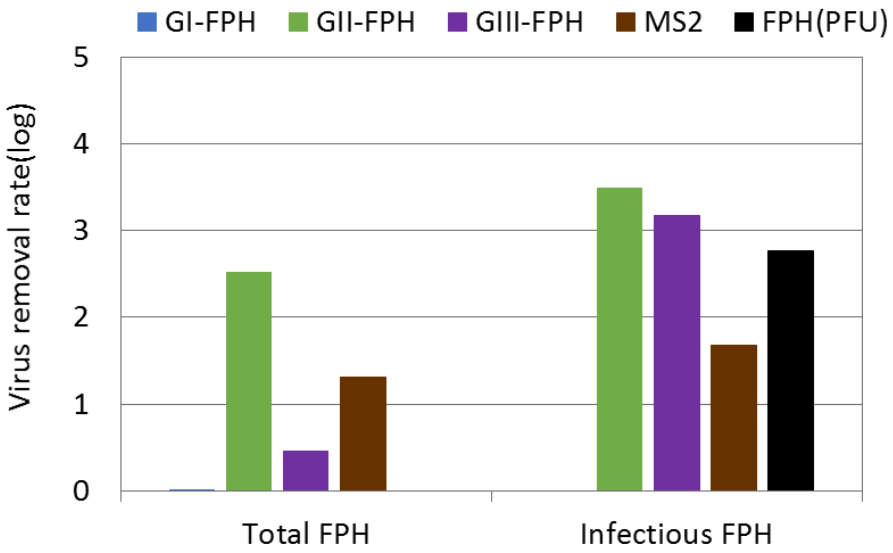


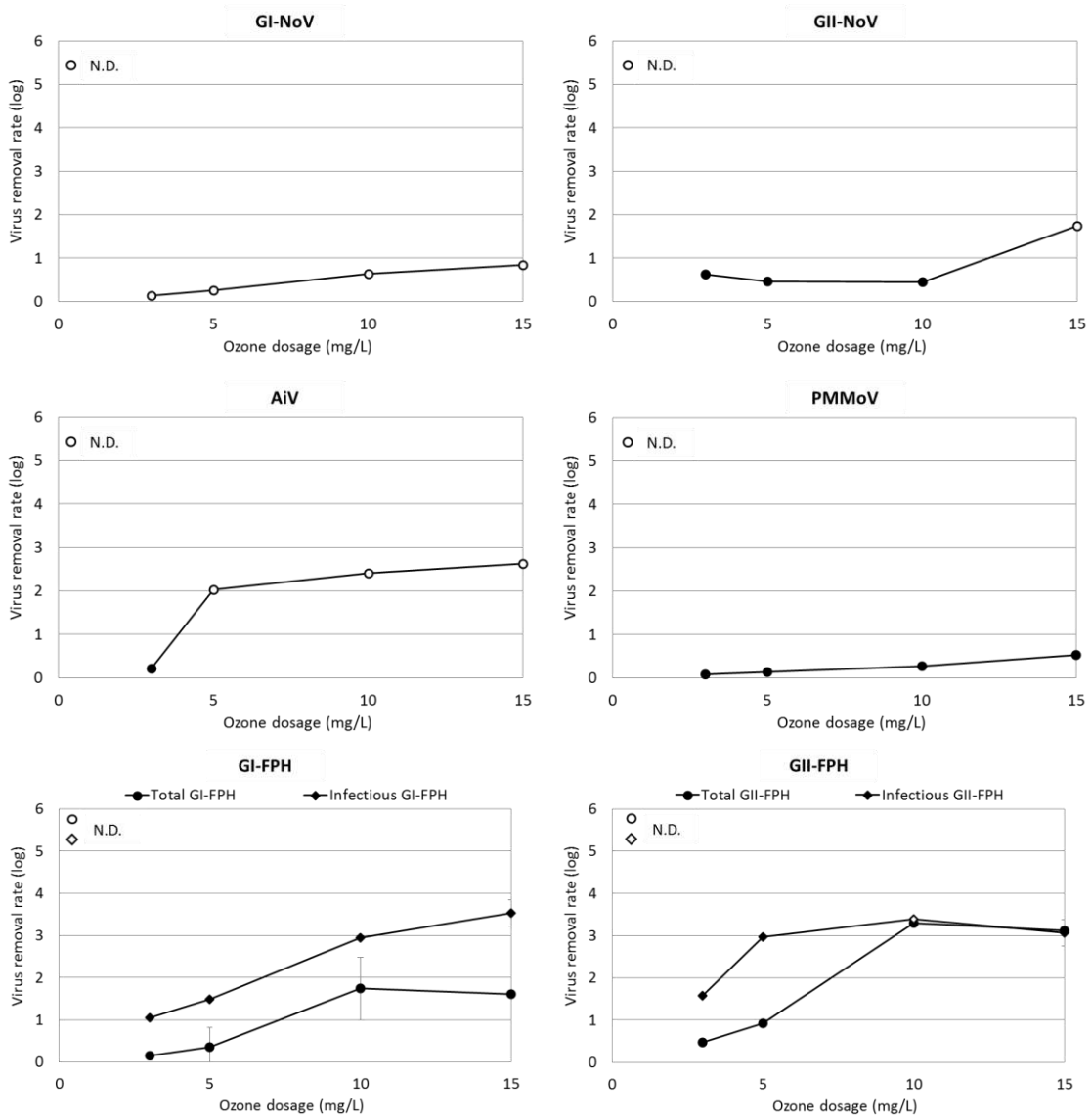
Figure 7.9 Total and infectious FPH and the spiked MS2 removal rate by PACl(150mg/L)+CMF for treating PE

Total and infectious GII-FPH removal rate was 2.5 and 3.5 log, respectively, under PACl(150mg/L)+CMF. On the other hand, both total and infectious GI-FPH was rarely removed. The removal rate of 0.5 log was observed at total GIII-FPH, while 3.2 log of infectious GIII-FPH was inactivated, indicating that GIII-FPH largely inactivated. As mentioned before, FPH could be inactivated by PACl, and among FPH genotypes, Qβ, a representative strain of GIII-FPH, was much readily inactivated by coagulants than MS2 (Shirasaki et al., 2009, Kreißel et al., 2014). The removal rate of total and infectious MS2 was 1.3 and 1.7 log, and it was higher than that of GI-FPH. The reason why there is the difference between the removal of MS2 and GI-FPH by PACl+CMF might be caused the difference in the concentration or the form of existence in source water as same with above explanation.

This result can provide valuable information regarding the removal and inactivation of indigenous viruses during ozonation and CMF, because there was no report in terms of virus removal by advanced treatment process for treating PE.

7.3.2.2.2 Post-ozonation

Figure 7.10 shows indigenous virus removal during post-ozonation for treating PACl+CMF permeate produced from PE. The experiment was triplicated. The horizontal axis and vertical axis represents O_3 consumption (mgO_3/mgC) and removal rate (log), respectively. N.D. represents that virus was not detected in ozonated water. The removal rate was calculated using detection limit of concentration if virus was not detected in ozonated water. In case of FPH removal rate, the removal rate calculated from FPH concentration quantified by RT-qPCR and by IC-RT-PCR was defined as total and infectious FPH removal rate, respectively.



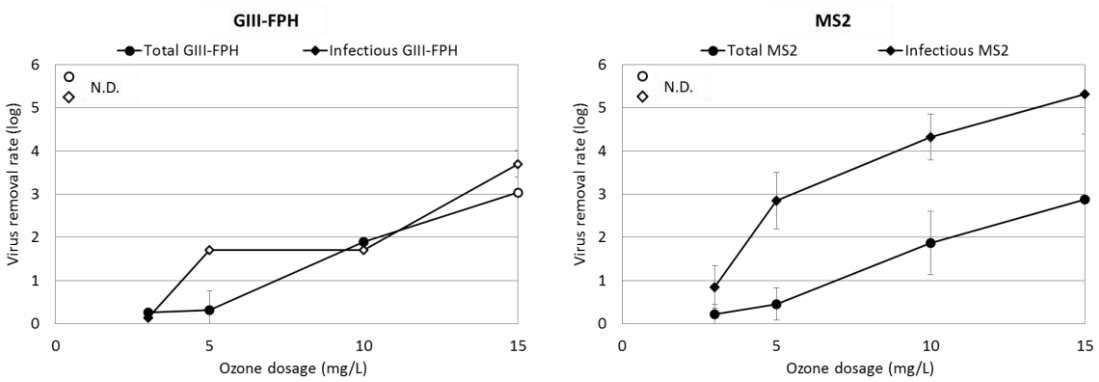


Figure 7.10 Indigenous virus and the spiked MS2 removal rates during post-ozonation (A dotted line represents trend line of the removal rate against mgO_3/mgC. White circle or diamond indicates that virus was not detected in ozonated water and represents the highest calculated removal rate.)

GI-NoV was detected at level of detection limit in CM permeate and it was N.D. in all ozonated water. The removal rate of AiV was 0.2 log under 0.22 mgO_3/mgC, and AiV was N.D. under ozone consumption higher than 0.35 mgO_3/mgC. The removal rate of GII-NoV was a maximum of 0.7 log under 0.22 to 0.60 mgO_3/mgC, and the slope (increases of removal rate against ozone consumption) was 1.43 log/mgO_3/mgC, indicating that GII-NoV was difficult to be removed compared to the other human enteric viruses. The removal rate of PMMoV was 0.6 log under 1 mgO_3/mgC, and the lowest slope was obtained (0.46 log/mgO_3/mgC) among examined indigenous viruses.

In case of FPH, the removal rate of total GI, GII and GIII-FPH was 0 to 2.5, 0.2 to 3.6 and 0 to 2.3 log, respectively, under 0.22 to 1 mgO_3/mgC, and the slope was 1.97, 3.66 and 3.07 log/mgO_3/mgC, respectively. The removal rate of infectious GI, GII and GIII-FPH was 1.3 to 3.9, 1.6 to > 4.4 and 0.1 to > 3.0 log, respectively, and the slope was 1.88, 1.33 and 2.87 log/mgO_3/mgC, respectively. In case of GI and GIII-FPH, quite similar slope value was observed between total removal rate and infectious removal rate. On the other hand, there was a difference in slope value of total GII-FPH removal and infectious GII-FPH. Compared to the other FPH genotypes, the slope of total GII-FPH removal was higher while the slope of infectious GII-FPH removal was similar. This result indicated that viral RNA of GII-FPH was readily degraded than the other FPH genotypes, while there was no significant difference in the inactivation among three FPH genotypes. The difference of the infectivity index between three FPH genotypes might be one of reasons. As same with SE, GII-FPH showed lowest infectivity index in CM permeate produced from PE (see Figure S6 (b) in the supplementary material). Therefore, the reason why the slope of total GII-FPH removal rate was highest might be explained by

the lowest infectivity index in CM permeate.

As similar with the results, GI-FPH tended to be difficult to be removed during post-ozonation. It can be confirmed more clearly through the comparison of mean removal rate (see Figure S8 in the supplementary material). In addition, 0.3 to 4.0 log of total MS2 removal rate and 1.2 to 5.0 log of infectious MS2 removal rate was observed under 0.2 to 1 mgO_3/mgC. The slope of total and infectious MS2 removal was 3.68 and 5.14 log/mgO_3/mgC, respectively. This result indicated that the removal rate of spiked MS2 was much higher than that of GI-FPH. Accordingly, the evaluation of virus removal performance during ozonation might be overestimated through MS2 spike test. In terms of total removal rate, GI-FPH showed much similar removal behavior with human enteric viruses such as GII-NoV, compared to spiked MS2. Consequently, it seems that GI-FPH has a potential as conservative surrogates.

7.3.3 The reconsideration on the applicability of reclaimed water based on GI-FPH removal

In this chapter, the applicability of reclaimed water was reconsidered using GI-FPH which showed the potential as conservative surrogates. Based on the result of infectious GI-FPH inactivation during O_3&CMF process, its virus removal rate was reevaluated and replotted in the graph of energy consumption in 4.3.3.

The inactivation of infectious GI-FPH during ozonation was estimated from trend line in Figure 7.4 and 7.10. In accordance with the result of infectious GI-FPH removal by PACl+CMF, the removal of indigenous virus by PACl+CMF was assumed as 3.4 and 1.0 log in SE and PE, respectively.

Figure 7.11 shows the applicability of reclaimed water based on the evaluation of virus removal performance of O_3&CMF process for treating (a) SE and (b) PE using MS2 and GI-FPH. This virus removal performance was evaluated by infectious MS2 and GI-FPH removal rate.

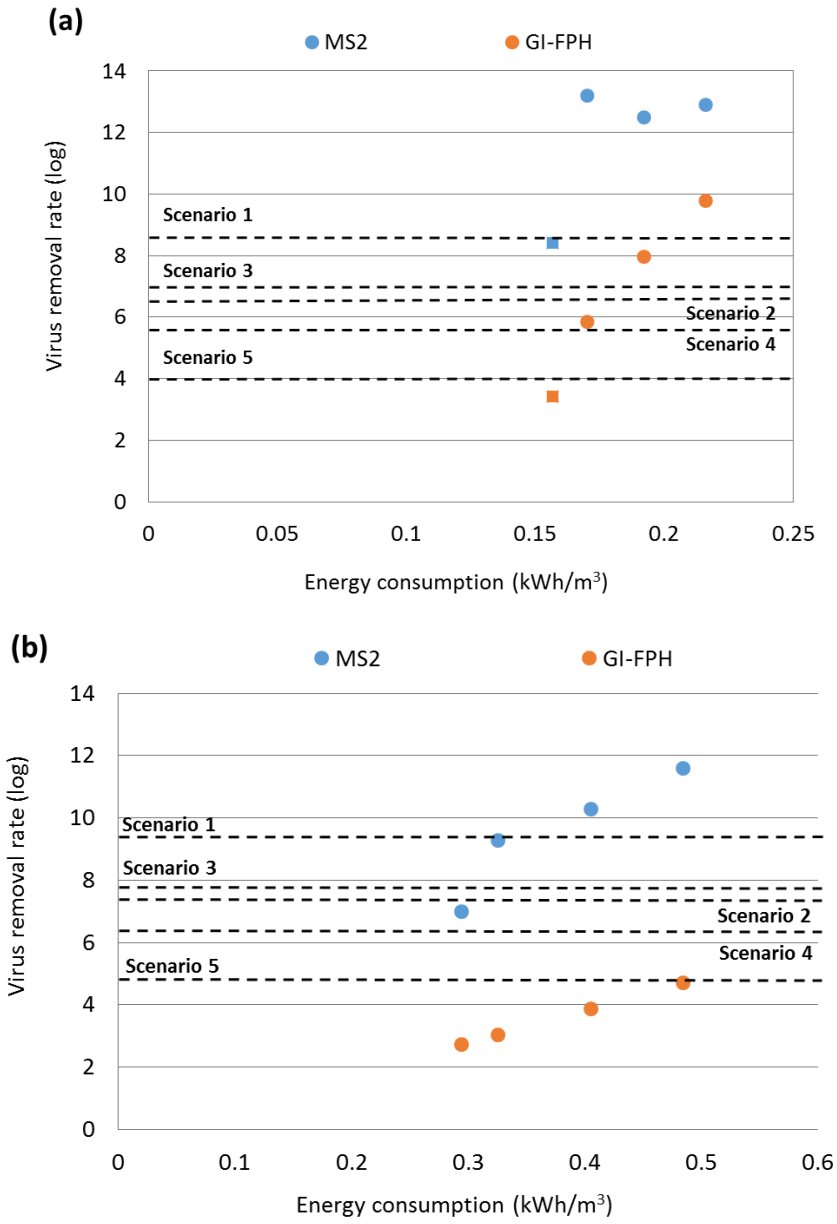


Figure 7.11 The applicability of reclaimed water based on the evaluation of virus removal performance of O_3&CMF process for treating (a) SE and (b) PE using infectious MS2 and GI-FPH (Square plot and circle plot indicates virus removal rate by PACl+CMF and by O_3&CMF process [O_3+PACl+CMF for SE or PACl+CMF+O_3 for PE], respectively)

In case of the evaluation of virus removal performance by O_3&CMF process for treating SE using MS2 as surrogates, a higher than 12 log of removal rate was obtained by incorporating pre-ozonation. This virus removal rate was higher than that required in all assumed scenarios. In case of the evaluation of virus removal performance using GI-

FPH as surrogates, however, 3.4 log of removal rate was observed by PACl+CMF. In addition, the removal rate of 5.9, 8.0 and 9.8 log was obtained by O_3+PACl+CMF under 2, 4 and 6 mg/L of ozone dosage, respectively. Accordingly, the virus removal rate by only PACl+CMF could not satisfy that required in all scenarios. Virus removal rate by O_3+PACl+CMF was satisfied that required in scenario 4 and 5 under 2 mg/L of ozone dosage, and it was satisfied that required in all scenarios under 6 mg/L of ozone dosage. In case of the evaluation of virus removal performance by O_3&CMF process for treating PE using MS2 as surrogates, virus removal rate required in all scenarios was satisfied by PACl(150mg/L)+CMF and higher than 5 mg/L of ozone dosage. In case of the evaluation virus removal performance using GI-FPH as surrogates, however, the removal rate of 2.7, 3.1, 3.9 and 4.7 log was observed under PACl(150mg/L)+CMF and 3, 5, 10 and 15 mg/L of ozone dosage, respectively. These removal rates could not satisfy that required in all scenarios. To satisfy the removal rate required in scenarios, thus, much higher ozone dosage was needed.

Consequently, it was found that the evaluation of virus removal performance through MS2 spike test was overestimated. There is a possibility that indigenous virus removal performance of O_3&CMF process was much smaller than that estimated from MS2 spike test. Accordingly, much more ozone or PACl dosage might be required to obtain the removal rate of indigenous virus as similar level with the spiked MS2 removal rate.

However, it was still unclear that the inactivation of human enteric viruses during ozonation. Thus, further study on the inactivation of human enteric viruses and the relationship with the inactivation of GI-FPH (to verify the applicability as surrogates) is needed.

7.4 Conclusions

In this chapter, the removal of indigenous virus in SE or PE by O_3&CMF process were investigated. Moreover, the removal of each genotype of infectious FPH was evaluated through quantitative genotyping using IC-RT-PCR assays. In addition, the obtained results were compared with that of MS2 spike test to investigate a difference between the removal performance of O_3&CMF process on indigenous viruses and MS2 artificially spiked.

The major conclusions can be drawn as follows:

1. Mean concentrations of GI-NoV, GII-NoV, AiV, PMMoV, GI, GII and GIII-FPH in SE were $1.5x10^4$, $4.3x10^4$, $5.3x10^3$, $2.2x10^7$, $2.6x10^4$, $5.7x10^5$ and $8.0x10^2$ copies/L,

respectively. By O_3&CMF process (6mg-O_3/L and 25mg-PACl/L), the concentration in CM permeates was detected at amounts near or below the detection limit (2 copies/L).

2. In pre-ozonation for treating SE, the removal rate of GI-NoV, GII-NoV, AiV and PMMoV was 0.4 ~ 2.5 log, 0 ~ 2.7 log, 0.1 ~ 1.6 log and 0 ~ 2.8log under 0.10 ~ 0.57 mgO_3/mgC, respectively. Moreover, the removal rate of indigenous FPH was 0.3 ~ 2.7 log under 0.10 ~ 0.57 mgO_3/mgC, regardless of the retention of their infectivity. The inactivation of infectious FPH was 1 ~ 3 log at 0.10 ~ 0.57 mgO_3/mgC. In PACl+CMF for treating SE, the removal rate of GI-NoV, GII-NoV, AiV and PMMoV was 2.2, 2.9, >2.6 and 3.6 log, respectively. By incorporating pre-ozonation (6mg-O_3/L), it increased to >3.8, >3.9, 2.6 and 6.6 log. As similar with the result of pre-ozonation, it was found that GI-FPH is difficult to be removed by PACl+CMF, compared to not only the other FPH but also human enteric viruses.

3. Mean concentrations GI-NoV, GII-NoV, AiV, PMMoV in PE were $2.1x10^4$, $2.8x10^5$, $3.7x10^4$ and $1.3x10^8$ copies/L, respectively. GI-NoV and AiV concentrations largely decreased by PACl+CMF, and they were N.D. in post-ozonated water. GII-NoV was still detected at the concentration of 10^3 copies/L in post-ozonated water. PMMoV, was detected at 10^4 ~ 10^6 copies/L and 10^3 ~10^5 copies/L in CM permeate (50mg/L of PACl) and post-ozonated water. Mean concentrations of GI, GII and GIII-FPH in PE were $1.2x10^6$, $8.4x10^7$ and $9.9x10^4$ copies/L, respectively. FPH concentrations were not significantly changed after PACl(50)+CMF. GI, GII and GIII-FPH were detected in range of 10^3 ~ 10^6, 10^2 ~ 10^5 and 10^2 ~ 10^5 copies/L, respectively, in CM permeate with PACl(150). FPH concentrations gradually decreased with increasing ozone dosage, and as a result all of three FPH genotypes were detected at a maximum level of 10^3 copies/L under 10 mg-O_3/L.

4. In O_3&CMF process for treating PE, the removal rate of GI-NoV, GII-NoV, AiV and PMMoV was 0.7 ~ 2, 1.5 ~ 3, 0.7 ~ 3 and 2 ~ 4 log, respectively, by PACl+CMF. In case of GI-FPH, both total and infectious GI-FPH was rarely removed. On the other hand, the removal rate of total and infectious MS2 was 1.3 and 1.7 log, indicating that it was higher than that of GI-FPH. In post-ozonation, the removal rate of GII-NoV was a maximum of 0.7 log under 0.22 ~ 0.60 mgO_3/mgC, and the slope was 1.43 log/mgO_3/mgC, indicating that GII-NoV was difficult to be removed compared to the other human enteric viruses. In case of FPH, the removal rate of total GI, GII and

GIII-FPH was 0 ~ 2.5, 0.2 ~ 3.6 and 0 ~ 2.3 log, respectively, under 0.22 ~ 1 mgO_3/mgC, respectively. Moreover, the removal rate of infectious GI, GII and GIII-FPH was 1.3 ~ 3.9, 1.6 ~ > 4.4 and 0.1 ~ > 3.0 log, respectively. GI-FPH tended to be difficult to be removed during post-ozonation. In addition, 0.3 ~ 4.0 log of total MS2 removal rate and 1.2 ~ 5.0 log of infectious MS2 removal rate was observed under 0.2 ~ 1 mgO_3/mgC. This result indicated that the removal rate of spiked MS2 was much higher than that of GI-FPH. Accordingly, the evaluation of virus removal performance during ozonation might be overestimated through MS2 spike test.

5. The applicability of reclaimed water was reconsidered using GI-FPH which showed the potential as a conservative surrogate. In case of the evaluation of virus removal performance of O_3&CMF process for treating SE using GI-FPH as surrogates, 3.4 log of removal rate was observed by PACl+CMF. Furthermore, the removal rate of 5.9, 8.0 and 9.8 log was obtained by O_3+PACl+CMF under 2, 4 and 6 mg/L of ozone dosage, respectively. Accordingly, the virus removal rate by only PACl+CMF could not satisfy that required in all scenario. In order to achieve virus removal rate required in all scenarios, 6 mg/L of ozone dosage was necessary. In case of the evaluation virus removal performance of O_3&CMF process for treating PE using GI-FPH as surrogates, the removal rate of 2.7, 3.1, 3.9 and 4.7 log was observed under PACl(150mg/L)+CMF and 3, 5, 10 and 15 mg/L of ozone dosage, respectively. These removal rates could not satisfy that required in all scenarios. To satisfy the removal rate required in scenarios, thus, much higher ozone dosage was needed. However, it was still unclear that the inactivation of human enteric viruses during ozonation. Thus, further study on the inactivation of human enteric viruses and the relationship with the inactivation of GI-FPH is needed.

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Chapter Ⅷ

Determination on a novel monitoring indicator considering virus removal and disinfection by-product formation

8.1 Introduction

By incorporating ozonation with ceramic membrane filtration, as demonstrated in the previous chapters, not only virus is effectively inactivated but also ceramic membrane fouling can be alleviated. However, disinfection by-products (DBPs) such as aldehydes can be formed, and their concentrations in reclaimed water depend on ozonation. Furthermore, the formation of DBPs and virus removal are variable even under constant ozone dosage because there are significantly influenced by the fluctuation of water quality. Therefore, it is difficult to ensure the reliability of water treatment performance, and also consistently provide reclaimed water which has hygienic safety. For this reason, water treatment monitoring systems, which make it possible to take action instantly when treatment fails, is required in order to ensure such reliability and to maintain full protection of public health.

Real-time monitoring systems has been studied in water treatment and water reclamation fields, and it has been proven that Excitation Emission Matrix Fluorescence Spectroscopy (EEM) has a potential as a novel monitoring technique (Reynolds and Ahmad, 1997; Ahmad and Reynolds, 1999; Saadi et al., 2006; Hudson et al., 2007; Hur et al., 2008; Henderson et al., 2009; Ishii and Boyer, 2012; Carstea et al., 2016). Hua et al. (2007) reported that the formation potentials of DBPs (trihalomethanes [THM] and N-nitrosodimethylamine [NDMA]) was highly related with fluorescence center at excitation: 290 ~ 310 nm/emission: 330 ~ 350 nm in drinking water treatment. According to Liu et

al. (2015), the formation of aldehydes during ozonation showed strong linear relationships with the relative changes of integrated fluorescence and therefore they reported a possibility of EEM as online monitoring indicator. However, there were only a few reports with regard to the monitoring of virus removal during ozonation, and most of all, an indicator which monitored both DBPs formation and virus removal at the same time has not been reported.

In this chapter, for this reason, the applicability of EEM as the monitoring indicator of DBPs formation and virus removal was investigated, and also EEM indicator was compared to conventional monitoring indicators such as dissolved ozone (DO_3) and UV_{254}. The applicability of EEM under the confined excitation and emission wavelength was also studied for the simplification of measurement.

8.2 Methods and materials

8.2.1 Ozonation experimental setup and methods

Ozonation experiment was conducted using both semi-batch ozone reactor and continuous bench scale reactors, and also followed experimental method, described in 3.2.4.1 and 4.2.3, respectively. The experiment was triplicated.

8.2.2 Analytical method

8.2.2.1 Water quality analysis

Water quality items were analyzed in accordance with the method described in 3.2.1

8.2.2.2 EEM analysis

EEM spectra were analyzed according to the method described in 5.2.2.1. The obtained EEM spectra were processed in accordance with fluorescence regional integration (FRI) method (Chen et al., 2003; Gerrity et al., 2012; Hernandez-Ruiz et al., 2012; Liu et al., 2015).

$$\emptyset_i = \sum_{Ex}\sum_{Em} I(\lambda_{Ex}\lambda_{Em})\Delta\lambda_{Ex}\Delta\lambda_{Em} \qquad \text{(Eq. 8.1)}$$

$$\Delta \mathrm{IF_i}/\mathrm{IF_{i_0}} = 1 - \emptyset_{i,O_3}/\emptyset_{i,0} \quad \text{(Eq. 8.2)}$$

Where,

$\emptyset_i$: Cumulative fluorescence intensity in EEM region "i"

i : EEM regions (Region I, II, III, IV and V)

$\Delta\lambda_{Ex}$: Excitation wavelength interval

$\Delta\lambda_{Em}$: Emission wavelength interval

$I(\lambda_{Ex}\lambda_{Em})$: Fluorescence intensity at each selected excitation-emission wavelength pair

$\Delta \mathrm{IF_i}/\mathrm{IF_{i_0}}$: Relative change of integrated EEM fluorescence in EEM region "i"

$\emptyset_{i,O_3}$: Cumulative fluorescence intensity after ozonation in EEM region "i"

$\emptyset_{i,0}$: Cumulative fluorescence intensity before ozonation in EEM region "i"

$\Delta \mathrm{IF}/\mathrm{IF_0}$: Relative change of integrated EEM fluorescence in the whole regions

8.3.2.3 Aldehyde analysis

Among the examined DBPs in this study, it was found that formaldehyde (FAH) is primarily formed during ozonation in chapter V. Therefore, the correlation between the formation DBPs and $\Delta \mathrm{IF}/\mathrm{IF_0}$ was investigated using aldehydes, especially FAH, in this chapter. Four aldehydes (FAH, acetaldehyde, butyraldehyde and propionaldehyde) were analyzed according to the method described in 5.2.2.2.

8.3.2.4 Virus analysis

In this chapter, bacteriophage MS2 (MS2) was selected as a surrogate of virus. MS2 was analyzed in accordance with the method described in 3.2.2

8.3 Results and discussion

8.3.1 Applicability of $\Delta \mathrm{IF}/\mathrm{IF_0}$ as a novel monitoring indicator

As described in 3.1.1, O_3 consumption (mgO_3/mgC) has a high correlation with MS2 removal rate during ozonation. Thus, the correlation between mgO_3/mgC and $\Delta IF/IF_0$ was firstly investigated.

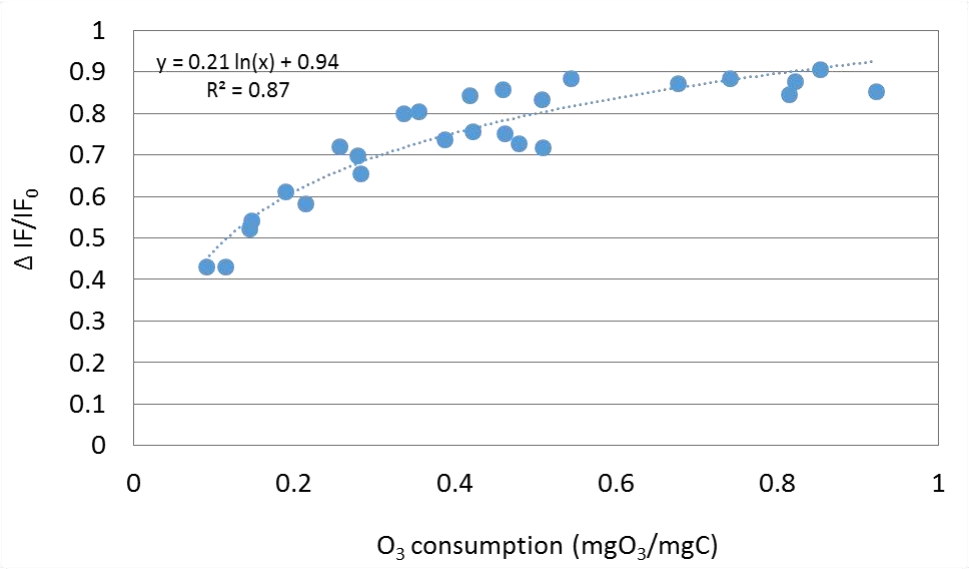

Figure 8.1 Correlations between O_3 consumption (mgO_3/mgC) and $\Delta IF/IF_0$

As a result, there was a logarithmical correlation (R^2=0.87) between mgO_3/mgC and $\Delta IF/IF_0$. It has been well documented that ozonation can degrade various organic compounds, especially aromatic compounds, and decrease the fluorescence intensity (Rodríguez et al., 2014; Wang et al., 2014).

From this result, it was expected that $\Delta IF/IF_0$ would have a correlation with virus removal. In addition, the technology to accurately monitor TOC or DOC value, needed for computation of mgO_3/mgC, has not been established, and therefore, $\Delta IF/IF_0$ has a potential to replace mgO_3/mgC.

The applicability of the relative change of integrated EEM was investigated as a monitoring indicator for the formation of DBPs and virus removal during ozonation.

Figure 8.2 shows the correlation of the formation DBPs or MS2 removal during ozonation with $\Delta IF/IF_0$.

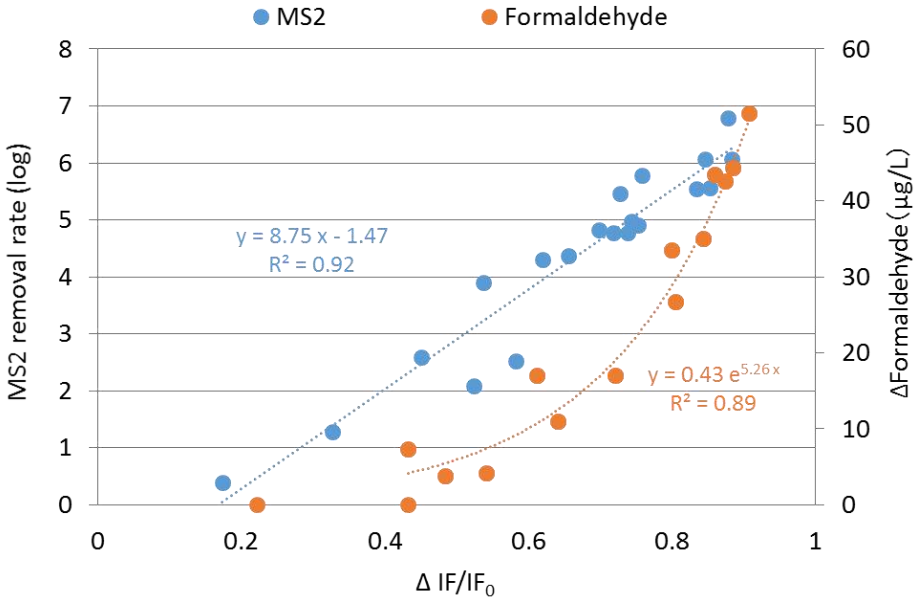

Figure 8.2 Correlations of MS2 removal and the formation of FAH with $\Delta IF/IF_0$

As shown in Figure 8.2, there was a high correlation of the formation of FAH or MS2 removal during ozonation with $\Delta IF/IF_0$. MS2 removal showed a linear correlation (R^2=0.92), and the formation of FAH showed an exponential correlation (R^2=0.89) with $\Delta IF/IF_0$.

Consequently, virus removal and the formation of DBPs could be predicted by monitoring $\Delta IF/IF_0$ without virus or DBPs analysis. This $\Delta IF/IF_0$ monitoring makes it possible to more promptly recognize and take an action when the treatment fails. Furthermore, it was also possible to operate ozonation and ceramic membrane filtration combination process with controlling ozone dosage to maintain $\Delta IF/IF_0$ stably. It was expected that such operation could be contributed to much stable supply of reclaimed water which has a hygienic safety.

8.3.2 Comparison with conventional indicators

It was found that $\Delta IF/IF_0$ has the potential as the monitoring indicator for the formation of FAH and virus removal. $\Delta IF/IF_0$ would be comparted to conventional indicators such as UV absorbance and DO_3. According to previous researches, ΔUV were strongly related to the removal of aromatic and non-aromatic compounds, and it is possible to predict the removal of them (Gerrity et al., 2012; Liu et al., 2012; Pisarenko et al., 2012).

Figure 8.3 shows the correlation of the relative change of UV_{254} with virus removal or the

formation of FAH. The horizontal axis represents the relative change of UV_{254} against initial UV_{254}, as similar with above $\Delta IF/IF_0$.

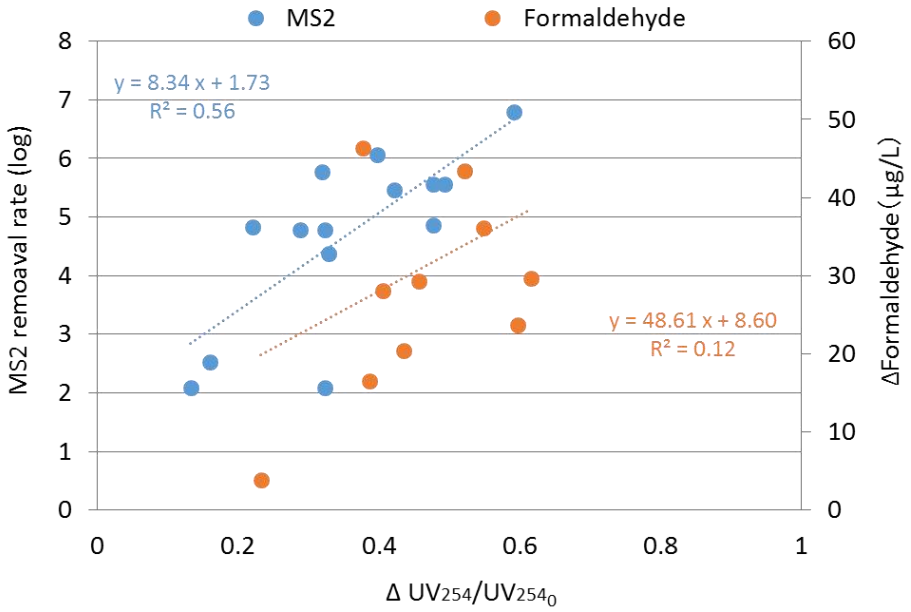

Figure 8.3 Correlations of MS2 removal and the formation of FAH with $\Delta UV_{254}/UV_{254_0}$

In both virus removal and the formation of FAH, there was a variation and they showed a low correlation (R^2=0.56 for virus removal; R^2=0.12 for the formation of FAH) with $\Delta UV_{254}/UV_{254_0}$. According to Liu et al. (2012), the strong correlation between the change of UV_{254} and the formation of aldehydes was observed. In this study, however, we conducted experiments several times using source water collected on different days, and as a result it was found that it is difficult to be normalized the formation of FAH under the variation of water quality. It means that it is difficult to predict the formation of FAH under various source water which have a different water quality only by $\Delta UV_{254}/UV_{254_0}$.

The control of ozone dosage by DO_3 allowed an appropriate supply of ozone and satisfactory level of the decomposition of organic matters even when the source water quality deteriorated (Aoki et al., 2009). Furthermore, it was mentioned that membrane fouling was effectively mitigated when ozone dosage set to be detected over certain level of DO_3, in Chapter IV. For this reason, DO_3 has a potential to be a monitoring indicator for operation of membrane filtration. In this session, the applicability of DO_3 as a monitoring indicator for treatment performance.

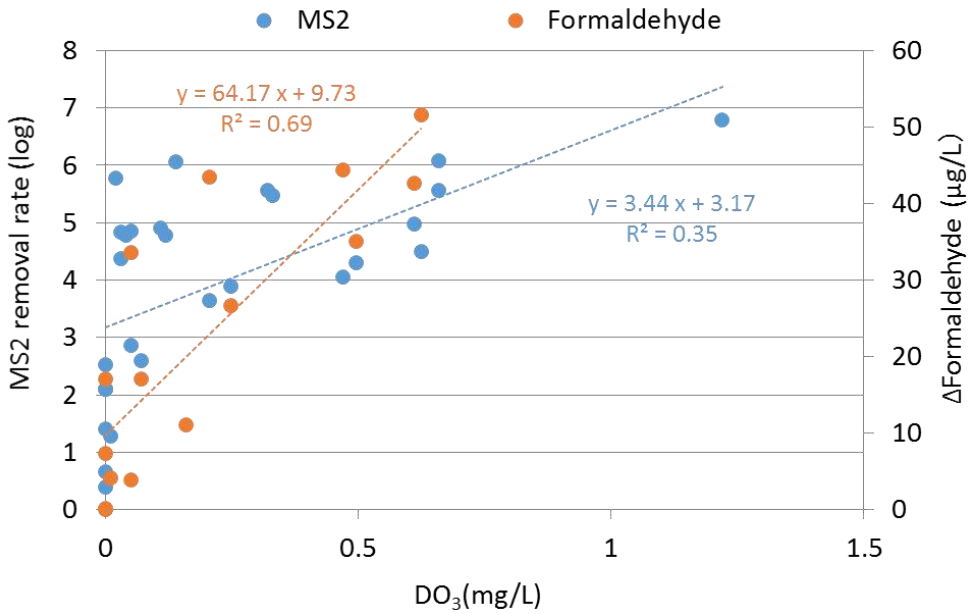

Figure 8.4 Correlations of MS2 removal and the formation of FAH with DO_3

As shown in Figure 8.4, there were the relatively high correlation (R^2=0.69) between the formation of FAH and DO_3, and MS2 removal showed the low correlation (R^2=0.35). However, there was a variation in both MS2 removal and the formation of FAH. Moreover, a maximum of 2.5 log of MS2 removal and 20 µg/L of FAH formation was observed although DO_3 was not detected. Accordingly, it was difficult to predict accurately virus removal and DBPs formation by DO_3 indicating that it was also difficult to ensure treatment performance.

Consequently, $\Delta IF/IF_0$ has better potential as the monitoring indicator compared to both DO_3 and $\Delta UV_{254}/UV_{254_0}$.

8.3.3 Simplification of EEM measurement

Although $\Delta IF/IF_0$ could predict virus removal and FAH formation, it was necessary to measure fluorescence intensity in all regions, and to compute integrated EEM fluorescence. It produces a time lag between measurement and an action when treatment falis. For this reason, simplification of EEM measurement was investigated in order to promptly recognize and take an action. In this session, the applicability of $\Delta IF/IF_0$ under confined excitation and emission wavelength was investigated as the monitoring indicator. To select wavelength which would be confined, EEM spectra was divided into five regions according to Chen et al. (2003), and cumulative fluorescence intensity in each EEM region was computed. On the basis of computation results, regions

where showed similar behavior with total $\Delta IF/IF_0$ regarding relative change of cumulative fluorescence intensity in each region was investigated (see Figure S9 in the supplementary material). As a result, the relative change of cumulative fluorescence intensity in region III and V showed most similar behavior with total $\Delta IF/IF_0$.

Accordingly, the measurement of wavelength was confined at 422 nm of emssion wavelength where a maximum peak observed in region V, and the correlation of virus removal or the formation of FAH with $\Delta IF_{Em422}/IF_{Em422_0}$ was investigated.

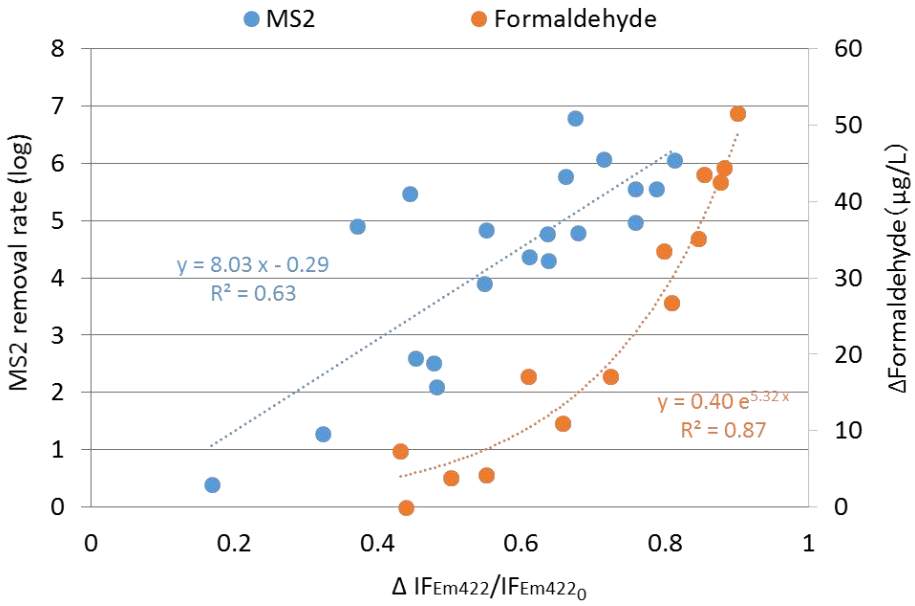

Figure 8.5 Correlations of MS2 removal and the formation of FAH with $\Delta IF_{Em422}/IF_{Em422_0}$

As a result, FAH formation showed a high correlation with $\Delta IF_{Em422}/IF_{Em422_0}$ (R^2=0.87), while the relatively low correlation was observed in MS2 removal (R^2=0.63). Most of all, the correlation of MS2 removal or FAH formation with $\Delta IF_{Em422}/IF_{Em422_0}$ was comparatively lower than that with $\Delta IF/IF_0$, indicating that the reliability of $\Delta IF_{Em422}/IF_{Em422_0}$ decreased as monitoring indicators.

The correlation of virus removal or the formation of FAH with $\Delta IF_{Ex250,Em280\sim480}/IF_{Ex250,Em280\sim480_0}$ was investigated under the measurement of wavelength confined at Ex 250 and Em 280 ~ 480 where a maximum peak was observed in region III.

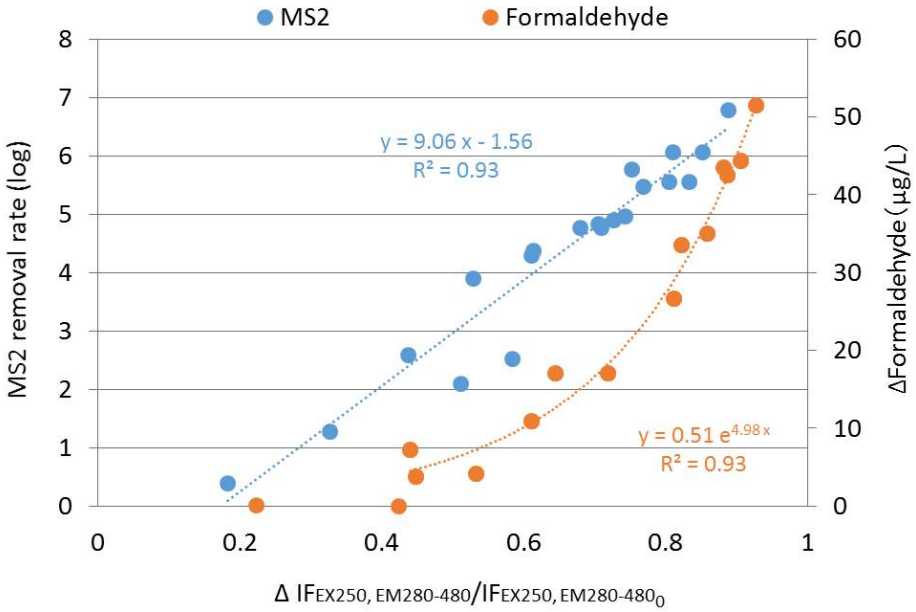

Figure 8.6 Correlations of MS2 removal and the formation of FAH with $\Delta IF_{Ex250,Em280\sim480}/IF_{Ex250,Em280\sim480_0}$

The high correlation of MS2 removal (R^2=0.93) or fomaldehyde formation (R^2=0.93) with $\Delta IF_{Ex250,Em280\sim480}/IF_{Ex250,Em280\sim480_0}$ was observed. Even though it has been well known that both region III and V was dominant fluorescence regions in wastewater (Baker et al., 2001; Liu et al., 2015; Cartea et al., 2016), $\Delta IF_{Ex250,Em280\sim480}/IF_{Ex250,Em280\sim480_0}$ which contains fluorescence peak in region III showed much higher correlation with virus removal than that of $\Delta IF_{Em422}/IF_{Em422_0}$ which include fluorescence peak in region V. It seems to be due to the difference in density of fluorescence peak. The fluorescence peak in region V which relatively spread out in broad area was difficult to be covered by Em 422. On the other hand, fluorescence peak in region III relatively concentrated in small area was easy to be covered by Ex 250 and Em 280 ~ 480.

Consequently, $\Delta IF/IF_0$ has a potential as monitoring indicator which could predict virus removal and DBPs formation. Moreover, it was possible to simplify measurement and promptly take an action due to shorten time lag by confining wavelength.
It was contributed to ensuring treatment performance under fluctuation of source water quality and providing hygienic safe reclaimed water. Further study on mechanistic interpretation of the correlation of virus removal and FAH formation with $\Delta IF/IF_0$ was needed.

8.3.4 Discussion on application methods of EEM as monitoring indicator

As mentioned above, relative change of integrated EEM fluorescence has a potential as the monitoring indicator.

EEM could be controlled to be minimized the risk of reclaimed water. Although ozonation could inactivate virus, DBPs were formed. It means that there was a trade-off relationship between virus removal and DBPs formation. Accordingly, virus infection risk could decreased during ozonation while lifetime cancer risk increased. Figure 8.7 shows the control of EEM from a viewpoint of trade-off relationship between virus removal and FAH formation during ozonation. The horizontal axis indicates $\Delta IF/IF_0$. The vertical axis in left side represents MS2 removal rate, and the vertical axis in right side indicates FAH formation. However, FAH formation value in the vertical axis was reversed against Figure 8.2.

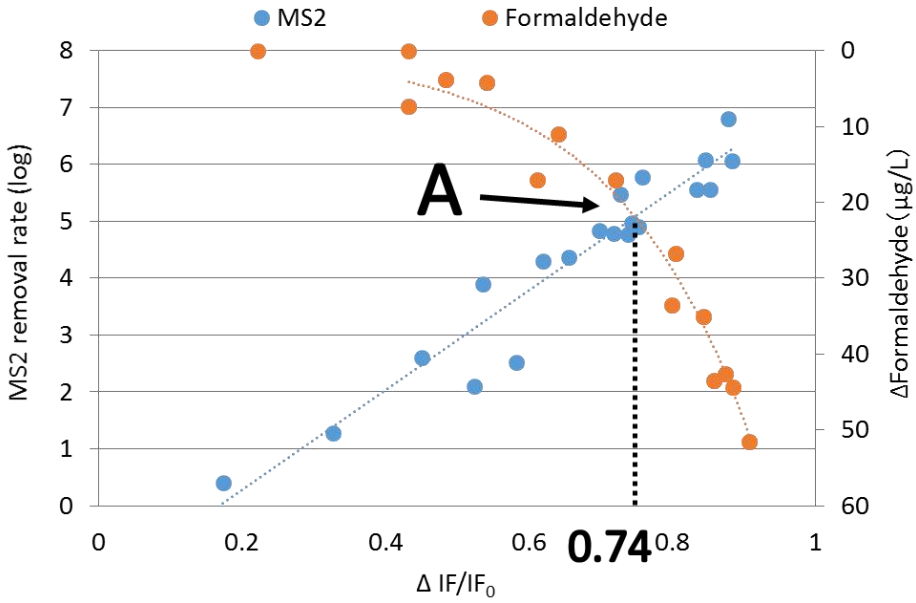

Figure 8.7 Control of EEM from a viewpoint of trade-off relationship between virus removal and FAH formation during ozonation

The point of intersection in Figure 8.7 (point A) might be the optimum point from a viewpoint of both maximizing virus removal and minimizing FAH formation. If IF/IF_0 were continuously controlled at 0.74 (point A), it could achieve that 5 log of virus removal rate and about 21 μg/L of FAH formation.

From the result of Chapter VI, however, it was revealed that lifetime cancer risk caused by FAH is not be a problem in all scenarios established in this study. On the other hand,

virus infection risk of reclaimed water produced by PACl+CMF was exceeded the acceptable risk of 10^{-5} when reclaimed was used for recreational impoundment (scenario 1). Therefore, virus removal rate should be monitored more carefully than FAH formation.

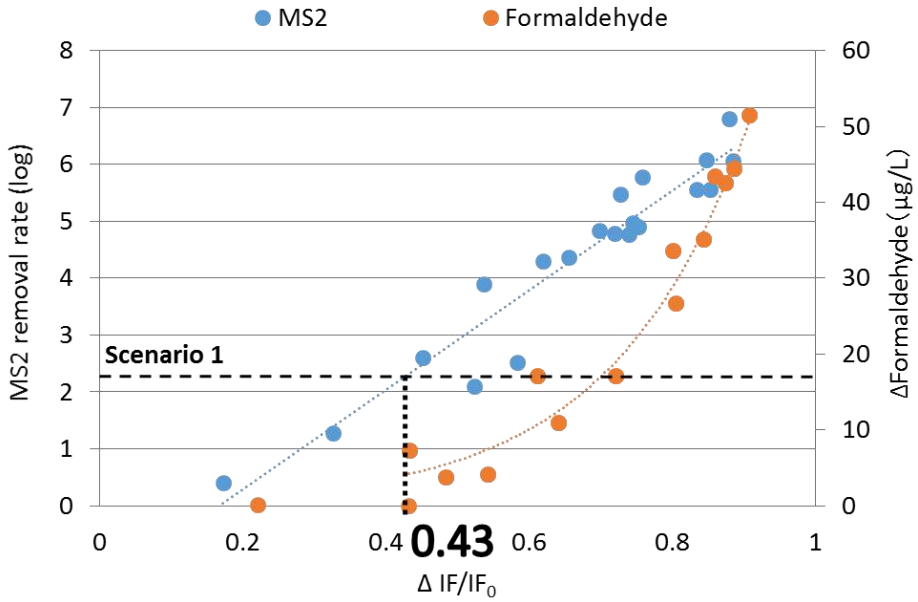

Figure 8.8 Control of EEM from a viewpoint of virus removal required in exposure scenario (Scenario 1)

This IF/IF_0 could be applied as not only the monitoring indicator, but also a control indicator to consistently achieve target virus removal depending on the uses of reclaimed water. Moreover, the formation of DBPs during ozonation was possible to be estimated by IF/IF_0. From the result of Chapter IV, for example, it was found that 6.7 to 8.4 log of virus removal rate is obtained by PACl(25mg/L)+CMF. Based on this result, about 2.3 log of virus removal was required during ozonation to achieve target removal rate in scenario 1 (8.3 log), if the removal rate by PACl+CMF were assumed at the minimum level of 6 log. If IF/IF_0 were continuously controlled at 0.43, about 2.3 log of virus removal rate could be obtained during ozonation, and 4.1 μg/L of FAH was formed. Even though the source water quality is wildly and constantly fluctuated, it was expected that target virus removal rate could be efficiently achieved, and also the concentration of DBPs in reclaimed water could be consistently maintained at the certain level when IF/IF_0 was used as the control indicator of ozone dosage.

8.4 Conclusions

In this chapter, the applicability of EEM as the monitoring indicator of DBPs formation and virus removal was investigated, and also EEM indicator was compared to conventional monitoring indicators such as DO_3 and UV_{254}. Moreover, the applicability of EEM under the confined excitation and emission wavelength was studied for the simplification of measurement.

The following conclusions can be drawn:

1. There was a high correlation of the formation of FAH or MS2 removal during ozonation with $\Delta IF/IF_0$. MS2 removal showed a linear correlation (R^2=0.92), and the formation of FAH showed an exponential correlation (R^2=0.89) with $\Delta IF/IF_0$.

2. It was found that $\Delta IF/IF_0$ has better potential as the monitoring indicator compared to both DO_3 and $\Delta UV_{254}/UV_{254_0}$. In addition, $\Delta IF/IF_0$ showed the high correlation with MS2 removal (R^2=0.93) or fomaldehyde formation (R^2=0.93) under wavelength confined at Ex 250 and Em 280 ~ 480 ($\Delta IF_{Ex250,Em280\sim480}/IF_{Ex250,Em280\sim480_0}$). It was possible to simplify measurement and promptly take an action due to shorten time lag by confining wavelength.

3. IF/IF_0 could be applied as not only the monitoring indicator, but also a control indicator to consistently achieve target virus removal depending on the uses of reclaimed water. If IF/IF_0 were used as the control indicator of ozone dosage, it was expected that target virus removal rate could be efficiently achieved, and also the concentration of DBPs in reclaimed water could be consistently maintained at the certain level.

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Chapter Ⅸ

Conclusions and recommendations

9.1 Conclusions

Water is one of the most essential natural resources for human. However, the water scarcity problem has been made worse due to increasing water demand and diminishing water resources caused by global population growth, urbanization and climate change since the Industrial Revolution. Wastewater, which has the consistent quantity and quality even under droughts and other climatic conditions, has been received an attention as alternative water resources. On the other hand, wastewater contains diverse contaminants including pathogens, chemicals and other toxins. The public health of reclaimed water users will be threaten if these contaminants were not eliminated adequately during water treatment. Moreover, water treatment process required enormous energy consumption to eliminate diverse contaminants and it is another problem which water reclamation was faced with. Thus, it is needed to develop the efficient treatment process in order to provide hygienically safe reclaimed water.

In this study, among the many treatment technologies, ozonation and ceramic membrane filtration combination process (O_3&CMF process) was selected as a treatment process for water reclamation. In this study, operation performance of ceramic membrane filtration (CMF) was evaluated. Moreover, not only both virus removal performance and disinfection by-products (DBPs) formation in O_3&CMF process was investigated, but also risk assessment of reclaimed water produced by O_3&CMF process was conducted. On basis of these comprehensive evaluations, this study aims to develop efficient O_3&CMF process considering the protection of public health.

The main findings of this study are summarized below by each chapter.

In Chapter III, the virus removal performance of both ozonation and coagulation was

evaluated through lab scale experiment, and also the amount of energy required to achieve target virus removal rate according to reclaimed water uses by O_3&CMF process was calculated. On the basis of these performance evaluation and the assessment of energy consumption, ultimately, the efficient process sequence in accordance with source water was decided prior to the continuous operation of O_3&CMF process in chapter IV. As a result, the similar MS2 removal rate was observed under same mgO_3/mgC during ozonation, even though source water had a different TOC or DOC value each other. The reduction of TOC by CMF was contributed to the decreases of the required ozone dosage. In coagulation, 3.6 ~ 6.5 log of MS2 removal rate was obtained under 10 ~ 30 mg/L of polyaluminium chloride (PACl) dosage in secondary effluent (SE), and 1.3 ~ 5.3 log of removal rate was observed under 50 ~ 150 mg/L of PACl dosage in primary effluent (PE). MS2 removal rate might be normalized roughly by mgPACl/mgC, but it needs to be conducted in SE and PE separately. MS2 removal rate by coagulation and sedimentation tended to decrease by pre-ozonation. In ozonated water, therefore, the much larger amount of PACl/TOC was required to obtain similar level with MS2 removal rate in SE. The hindrance of MS2 coagulation by pre-ozonation was attribute to the increases of negative charge, and this increase seems to be due to the change of polarity in ozonated water. However, the hindrance of MS2 coagulation was compensated by MS2 inactivation capability of pre-ozonation. From the result of the calculation of energy consumption, it was expected that ozonation followed by CMF (O_3+PACl+CMF) and CMF followed by ozonation (PACl+CMF+O_3) was efficient process for treating SE and PE, respectively.

In Chapter IV, both operation performance and virus removal performance of O_3&CMF process was evaluated through continuous operation. The higher than 12 log of MS2 removal rate was observed in O_3+PACl+CMF for treating SE. In case of operational performance, pre-ozonation successfully mitigate membrane fouling, and as a result the chemical enhanced backwashing (CEB) interval was extended from 24 to 345 h with increasing ozone dosage from 0 to 6 mg/L. In PACl+CMF+O_3 for treating PE, 6 log of MS2 removal rate was obtained at 150 mg-PACl/L, and 3.6 and 5 log of MS2 removal rate was obtained by 0.5 and 0.7 mgO_3/mgC of post-ozonation, repectively. In case of operational performance, the CEB interval was estimated as 60 and 180 h under the condition of 50 and 150 mgPACl/L, respectively. In terms of energy consumption, 0.157 ~ 0.216 kWh/m^3 was obtained in O_3+PACl+CMF for treating SE. Although energy consumption was slightly increased, higher MS2 removal rate than that required in all scenarios was achieved by incorporating pre-ozonaion. In case of PACl+CMF+O_3 for

treating PE, 0.198 ~ 0.484 kWh/m^3 of energy consumption was obtained. It was found that the relatively high PACl dosage (150 mg/L) was more efficient from energy aspect, compared to the condition of low PACl dosage (50 and 100 mg/L). As a result, MS2 removal required in all scenarios was satisfied by PACl(150mg/L)+CMF and post-ozonation (> 5 mg/L).

In Chapter V, DBPs formation during O_3&CMF was investigated. In addition, the effect on not only the removal of DBPs but also CMF caused by adding biological activated carbon (BAC) to O_3&CMF process were investigated. Ozonation formed primarily formaldehyde (FAH) up to a level of concentration which could be a problem on drinking water regulation established by Japan. Although a little amount of *N*-nitrosodimethylamine (NDMA) and chloroform (TCM) was formed, their formation potential (FP) was dramatically reduced during ozonation. It was expected that reclaimed water produced from PE by PACl+CMF+O_3 could contain both FAH and TCM at concentrations of several hundreds of μg/L. In addition, even though ozonation could reduce FP of NDMA, approximately 1000 ng/L of NDMA was remained in reclaimed water after chlorination. In case of PE, therefore, it is recommended that the utilization of reclaimed water should be restricted to the use which has less possibility to be exposed to users.

BAC could reduce DBPs examined in this study. Especially, FAH which is well known as easily biodegradable compounds was effectively removed through BAC. In addition, the extension of empty bed contact time (EBCT) can improve the removal of both DBPs and their FP. However, the leakage of microorganism such as general bacteria and heterotrophic bacteria from BAC was found. Moreover, the increasing tendency of the peak intensity corresponding to SMP-like materials, which was considered as major foulants, was observed in Excitation Emission Matrix Fluorescence Spectroscopy (EEM) spectra. These phenomenon may cause accelerated membrane fouling. Indeed, not only higher peak intensity corresponding to SMP-like materials, but also greater protein and carbohydrate content were detected in extracted foulants from ceramic membrane of with BAC, compared to that of without BAC. BAC has a potential as one of options as additional treatment in case that DBPs should rigorously be controlled depending on the use of reclaimed water. However, the optimization of operation condition such as EBCT is required to minimize the negative effect on ceramic membrane filtration by the addition of BAC.

In Chapter VI, the assessment of both virus infection risk and lifetime cancer risk was

conducted depending on the uses of reclaimed water. In addition, the applicability of reclaimed water for several uses was evaluated based on risk assessment. Virus infection risk from using recycled water produced by P1 met acceptable risk (10^{-6} DALYpppy) in scenario 2 ~ 6, but the 95th percentile virus infection risk was higher than the acceptable risk in scenario 1. It indicated that P1 was insufficient as treatment process when the uses of reclaimed water were recreational impoundment (scenario 1). However, infection risk due to exposure to viruses in recycled water produced by P2 met acceptable risk in all exposure scenarios. In scenario 1 ~ 5, lifetime cancer risk of 10^{-6} to 10^{-11} was obtained, and it was much smaller than 10^{-5} of acceptable risk. Therefore, lifetime cancer risk caused by DBPs in reclaimed water not seem to be a problem in scenario 1 ~ 5. In scenario 6, however, NDMA cancer risk was higher than 10^{-5}. This NDMA cancer risk could decrease to below than 10^{-5} by adding BAC treatment. Therefore, O_3&CMF process with BAC was recommended to reduce NDMA cancer risk.

There was no significant difference between virus infection risk in recycled water produced by P2-2, P2-4 and P2-6. Lifetime cancer risk caused by FAH increased by about 10^{-1} with increasing ozone dosage, while there are no significant increases in lifetime cancer risk caused by NDMA or TCM. Compared to reclaimed water produced by P1, the decreases of virus infection risk by incorporating ozonation was larger than the increases of lifetime cancer risk. Accordingly, it was possible to extend the uses of reclaimed water by incorporating ozonation regardless of the condition of ozone dosage. The reclaimed water produced by P1 can be used for scenario 2 ~ 5. In case of the reclaimed water produced by P2, it can be used for scenario 1 ~ 5 regardless of ozone dosage tested in this study. However, it was unable to be used for scenario 6 because lifetime cancer risk exceeded acceptable risk. For using reclaimed water as the uses of scenario 6, it was necessary to be applied P3 which contains BAC treatment to reduce lifetime cancer risk.

In Chapter VII, the removal of both indigenous virus and F-specific RNA phage (FPH) in wastewater by O_3&CMF process were investigated. Furthermore, the removal of each genotype of infectious FPH was evaluated through quantitative genotyping using IC-RT-PCR assays. In addition, the obtained results were compared with that of MS2 spike test to investigate a difference between the removal performance of O_3&CMF process on indigenous viruses and MS2 artificially spiked. Mean concentrations of GI-NoV, GII-NoV, AiV, PMMoV, GI, GII and GIII-FPH in SE were $1.5x10^4$, $4.3x10^4$, $5.3x10^3$, $2.2x10^7$, $2.6x10^4$, $5.7x10^5$ and $8.0x10^2$ copies/L, respectively. By O_3&CMF process (6mg-O_3/L and 25mg-PACl/L), the concentration in CM permeates was detected at amounts near or below the

detection limit (2 copies/L). In pre-ozonation for treating SE, the removal rate of GI-NoV, GII-NoV, AiV and PMMoV was 0.4 ~ 2.5 log, 0 ~ 2.7 log, 0.1 ~ 1.6 log and 0 ~ 2.8log under 0.10 ~ 0.57 mgO_3/mgC, respectively. Moreover, the removal rate of indigenous FPH was 0.3 ~ 2.7 log under 0.10 ~ 0.57 mgO_3/mgC, regardless of the retention of their infectivity. The inactivation of infectious FPH was 1 ~ 3 log at 0.10 ~ 0.57 mgO_3/mgC. In PACl+CMF for treating SE, the removal rate of GI-NoV, GII-NoV, AiV and PMMoV was 2.2, 2.9, >2.6 and 3.6 log, respectively. By incorporating pre-ozonation (6mg-O_3/L), it increased to >3.8, >3.9, 2.6 and 6.6 log. It was found that GI-FPH is difficult to be removed by PACl+CMF, compared to not only the other FPH but also human enteric viruses.

Mean concentrations GI-NoV, GII-NoV, AiV, PMMoV in PE were $2.1x10^4$, $2.8x10^5$, $3.7x10^4$ and $1.3x10^8$ copies/L, respectively. GII-NoV was still detected at the concentration of 10^3 copies/L in post-ozonated water. PMMoV, was detected at 10^4 ~ 10^6 copies/L and 10^3 ~10^5 copies/L in CM permeate (50mg/L of PACl) and post-ozonated water. Mean concentrations of GI, GII and GIII-FPH in PE were $1.2x10^6$, $8.4x10^7$ and $9.9x10^4$ copies/L, respectively. FPH concentrations were not significantly changed after PACl(50)+CMF. FPH concentrations gradually decreased with increasing ozone dosage, and as a result all of three FPH genotypes were detected at a maximum level of 10^3 copies/L under 10 mg-O_3/L. In O_3&CMF process for treating PE, the removal rate of GI-NoV, GII-NoV, AiV and PMMoV was 0.7 ~ 2, 1.5 ~ 3, 0.7 ~ 3 and 2 ~ 4 log, respectively, by PACl+CMF. In case of GI-FPH, both total and infectious GI-FPH was rarely removed. On the other hand, the removal rate of total and infectious MS2 was 1.3 and 1.7 log, indicating that it was higher than that of GI-FPH. In post-ozonation, the removal rate of GII-NoV was a maximum of 0.7 log under 0.22 ~ 0.60 mgO_3/mgC, indicating that GII-NoV was difficult to be removed compared to the other human enteric viruses. In case of FPH, the removal rate of total GI, GII and GIII-FPH was 0 ~ 2.5, 0.2 ~ 3.6 and 0 ~ 2.3 log, respectively, under 0.22 ~ 1 mgO_3/mgC, respectively. Moreover, the removal rate of infectious GI, GII and GIII-FPH was 1.3 ~ 3.9, 1.6 ~ > 4.4 and 0.1 ~ > 3.0 log, respectively. GI-FPH tended to be difficult to be removed during post-ozonation. In addition, 0.3 ~ 4.0 log of total MS2 removal rate and 1.2 ~ 5.0 log of infectious MS2 removal rate was observed under 0.2 ~ 1 mgO_3/mgC. This result indicated that the removal rate of spiked MS2 was much higher than that of GI-FPH. Accordingly, the evaluation of virus removal performance during ozonation might be overestimated through MS2 spike test. The applicability of reclaimed water was reconsidered using GI-FPH which showed the potential as a conservative surrogate. In case of the evaluation of virus removal performance of O_3&CMF process for treating SE using GI-FPH as surrogates, 3.4 log of

removal rate was observed by PACl+CMF. Furthermore, the removal rate of 5.9, 8.0 and 9.8 log was obtained by O_3+PACl+CMF under 2, 4 and 6 mg/L of ozone dosage, respectively. Accordingly, the virus removal rate by only PACl+CMF could not satisfy that required in all scenario. In order to achieve virus removal rate required in all scenarios, 6 mg/L of ozone dosage was necessary. In case of the evaluation virus removal performance of O_3&CMF process for treating PE using GI-FPH as surrogates, the removal rate of 2.7, 3.1, 3.9 and 4.7 log was observed under PACl(150mg/L)+CMF and 3, 5, 10 and 15 mg/L of ozone dosage, respectively. These removal rates could not satisfy that required in all scenarios. To satisfy the removal rate required in scenarios, thus, much higher ozone dosage was needed.

In Chapter VIII, the applicability of EEM as the monitoring indicator of DBPs formation and virus removal was investigated, and also EEM indicator was compared to conventional monitoring indicators such as DO_3 and UV_{254}. The applicability of EEM under the confined excitation and emission wavelength was also studied for the simplification of measurement. As a result, there was a high correlation of the formation of FAH or MS2 removal during ozonation with $\Delta IF/IF_0$. MS2 removal showed a linear correlation (R^2=0.92), and the formation of FAH showed an exponential correlation (R^2=0.89) with $\Delta IF/IF_0$. It was found that $\Delta IF/IF_0$ has better potential as the monitoring indicator compared to both DO_3 and $\Delta UV_{254}/UV_{254_0}$. In addition, $\Delta IF/IF_0$ showed the high correlation with MS2 removal (R^2=0.93) or fomaldehyde formation (R^2=0.93) under wavelength confined at Ex 250 and Em 280 ~ 480 ($\Delta IF_{Ex250,Em280\sim480}/IF_{Ex250,Em280\sim480_0}$). It was possible to simplify measurement and promptly take an action due to shorten time lag by confining wavelength. IF/IF_0 could be applied as not only the monitoring indicator, but also a control indicator to consistently achieve target virus removal depending on the uses of reclaimed water. If IF/IF_0 were used as the control indicator of ozone dosage, it was expected that target virus removal rate could be efficiently achieved, and also the concentration of DBPs in reclaimed water could be consistently maintained at the certain level.

9.2 Recommendations for future study

1. In this study, operational performance of O_3&CMF process was evaluated through the continuous operation for approximately 40 days. On basis of the result obtained in this study, the stability of operational performance should be evaluated using full scale of ceramic membrane during much longer operation. Moreover, the assessment of energy consumption of O_3&CMF process was needed considering much various factors such as the energy consumption required for sludge treatment.

2. In this study, it was found that the evaluation of virus removal performance through MS2 spike test might be overestimated. There was a possibility that the removal rate of indigenous virus was much smaller than that of MS2 spiked, and GI-FPH showed the potential as a conservative surrogate. However, it was still unclear that the inactivation of human enteric viruses during O_3&CMF process. Thus, further study on the inactivation of human enteric viruses and the relationship with the inactivation of GI-FPH is needed, and it can contribute to much more practical evaluation of virus removal performance and risk assessment of reclaimed water.

Supplementary material

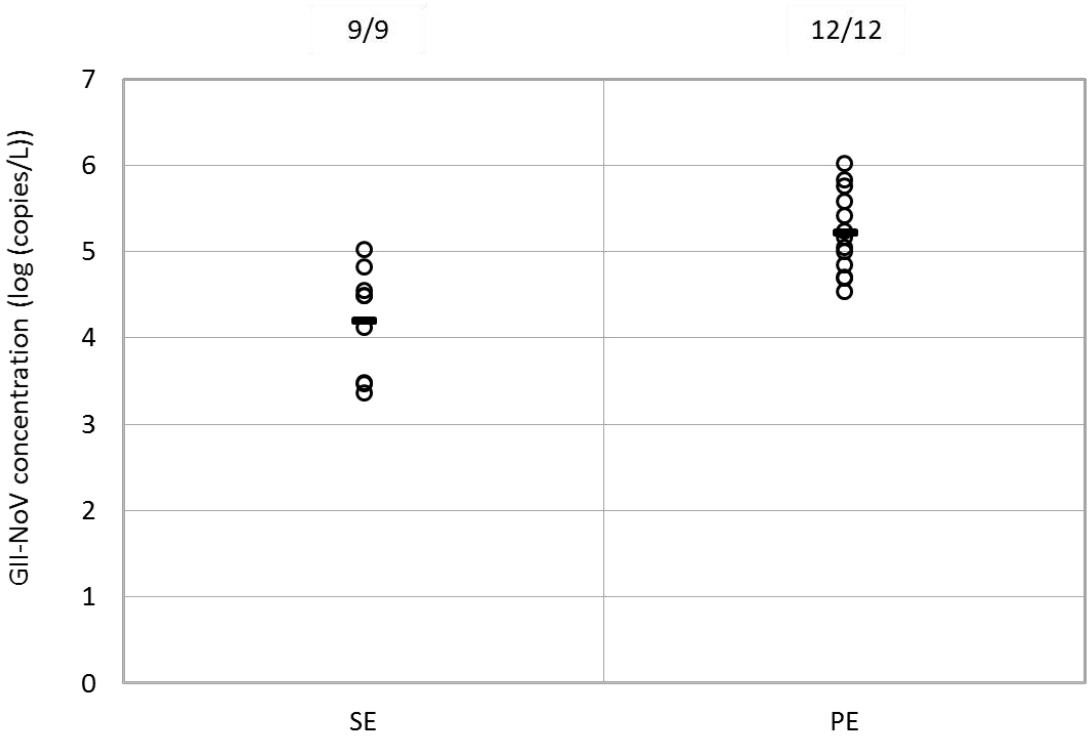

Figure S1 GII-NoV concentrations in wastewater (SE : secondary effluent, PE : primary effluent. Bar plot indicates the mean concentration of GII-NoV. Numbers above each graph item indicate the number of positive samples/total samples.)

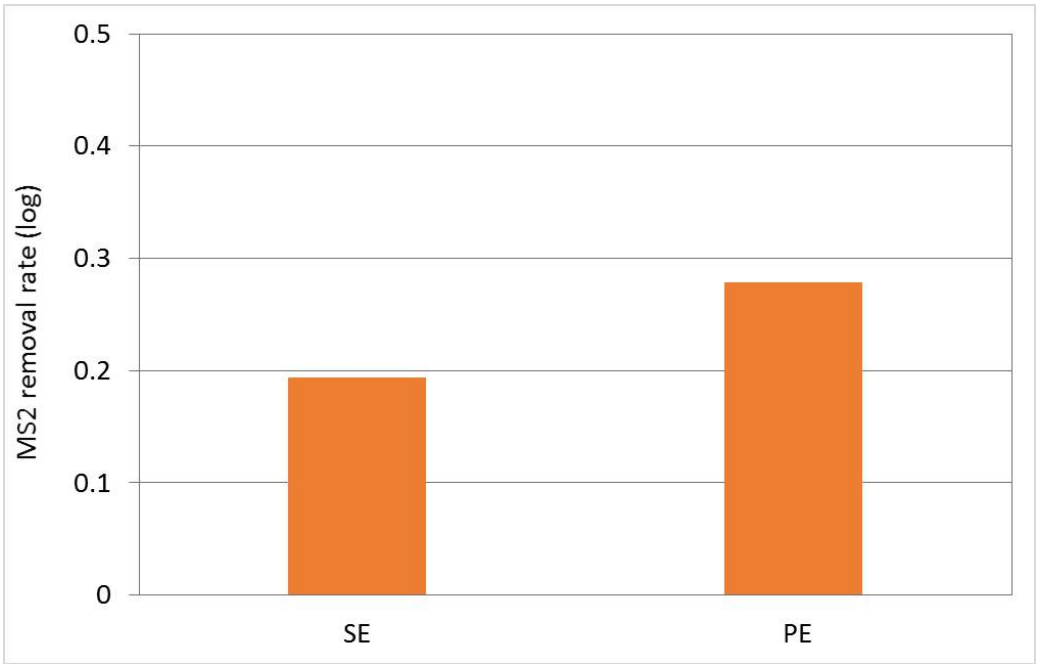

Figure S2 MS2 removal rate by CMF without coagulation (SE : secondary effluent, PE : primary effluent)

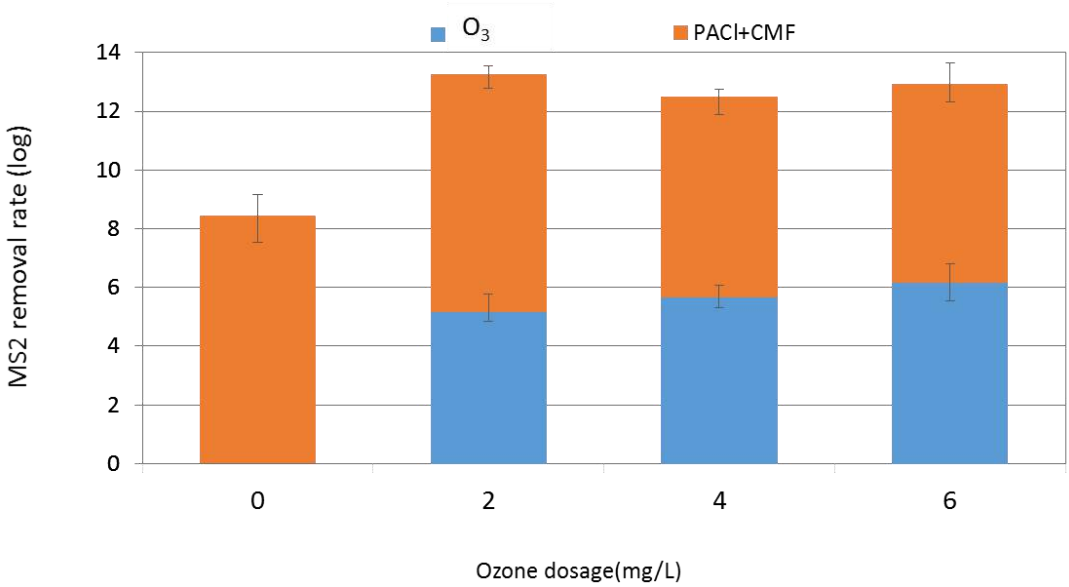

Figure S3 MS2 removal by O_3+PACl+CMF for treating SE (O_3 : ozonation, PACl : coagulation, CMF : ceramic membrane filtration, SE : secondary effluent. The value represents mean MS2 removal rate, and error bars indicate the standard deviation)

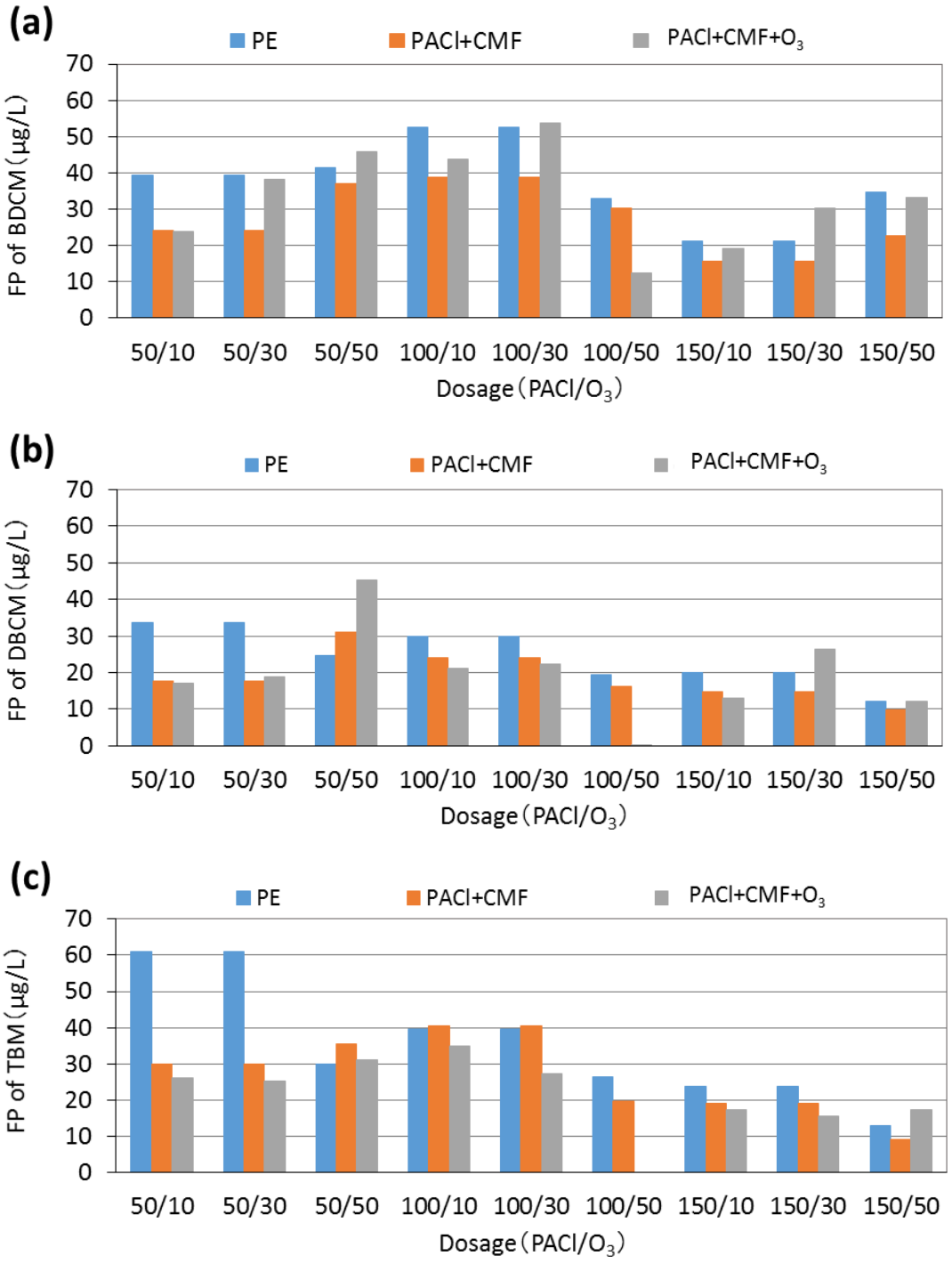

Figure S4 Formation potential of (a) BDCM, (b) DBCM and (c) TBM in PACl+CMF+ O_3 for treating PE (FP: formation potential, PE : primary effluent, PACl : coagulation, CMF : ceramic membrane filtration, O_3 : ozonation, BDCM : bromodichloromethane, DBCM : dibromochloromethane, TBM : bromoform)

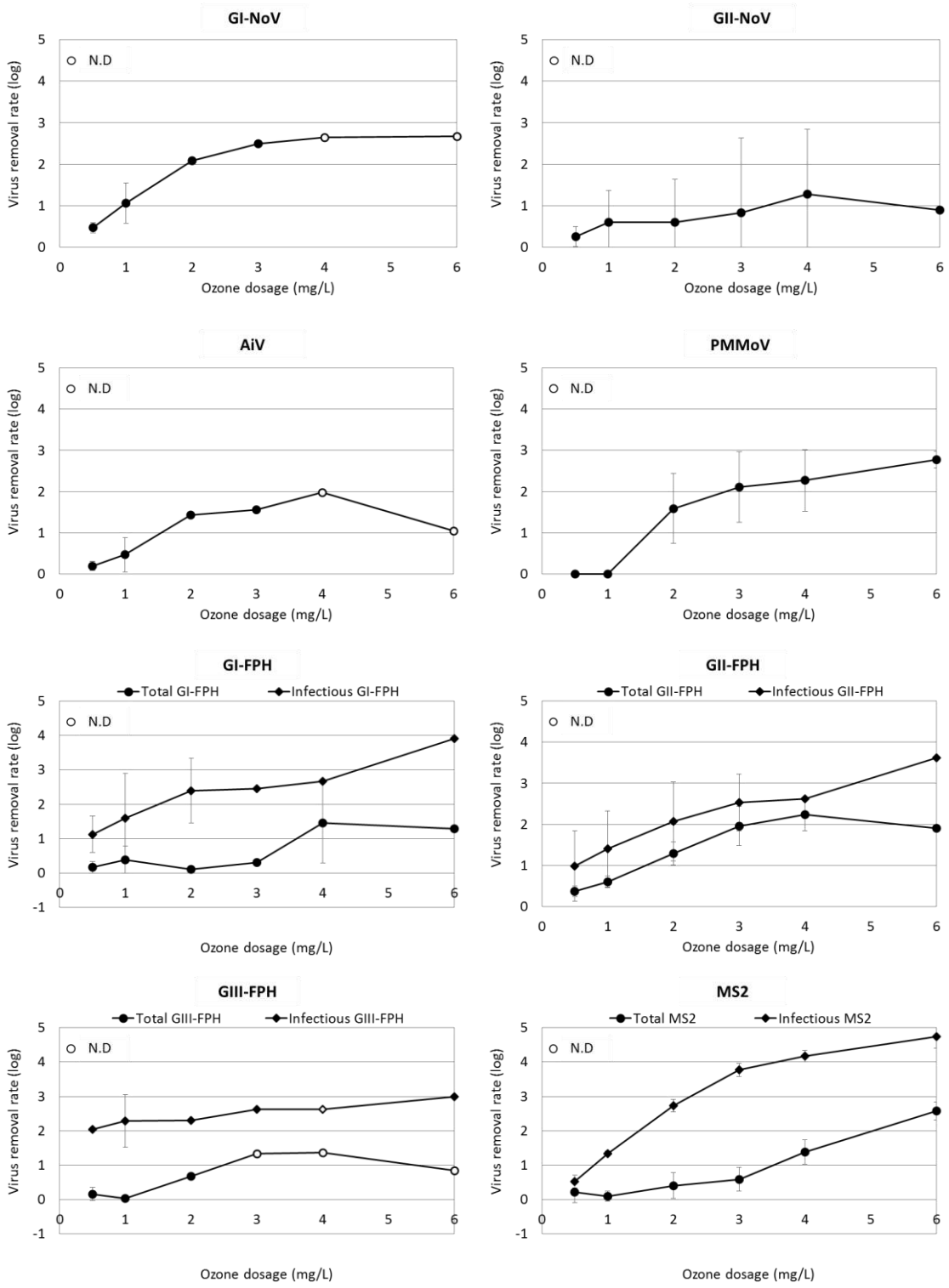

Figure S5 Mean removal rate of indigenous virus and the spiked MS2 during pre-ozonation (Source water was secondary effluent. White circle or diamond indicates that virus was not detected in ozonated water. Error bar represent the standard deviation)

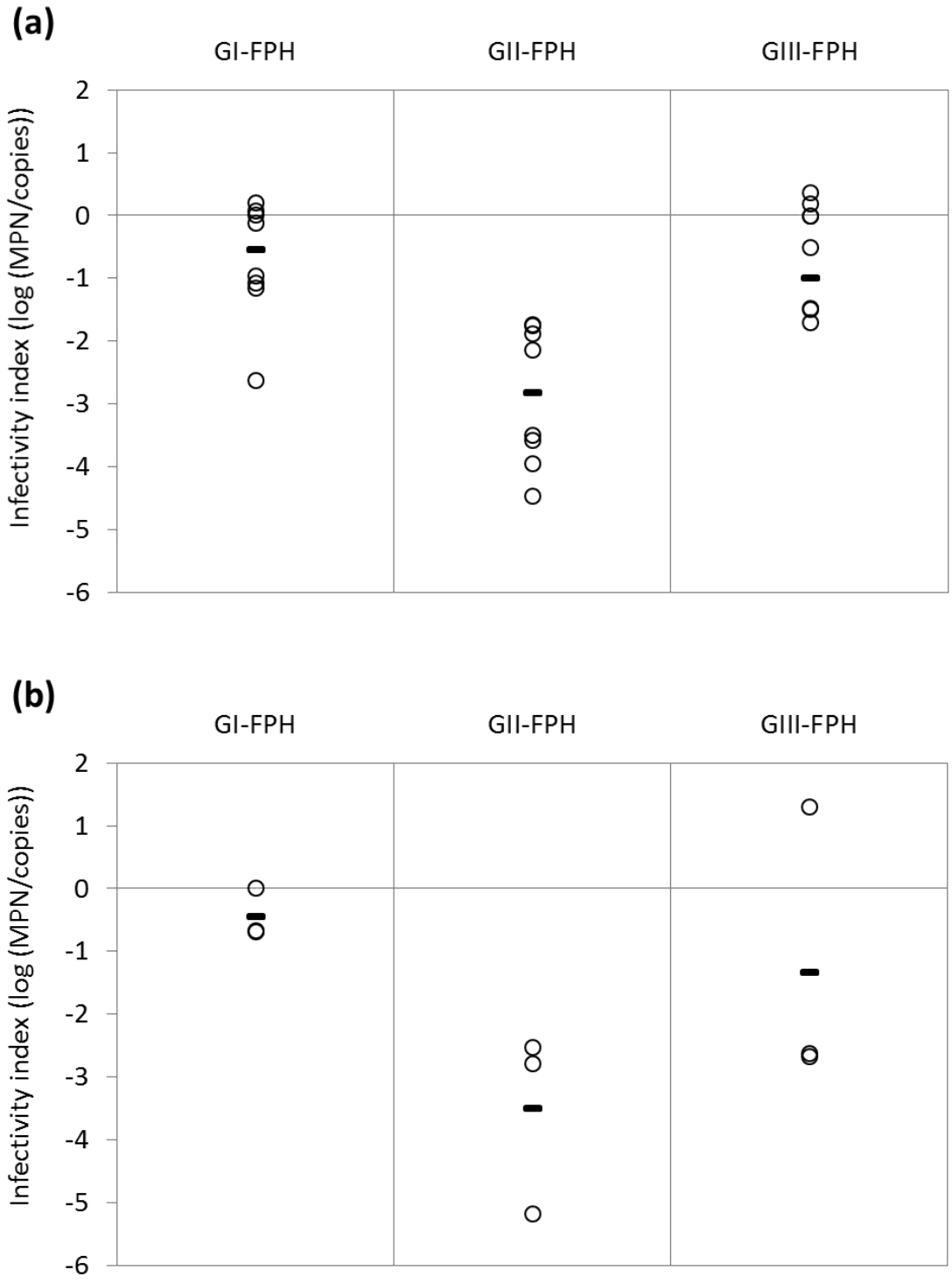

Figure S6 Infectivity index of FPH in (a) SE and in (b) ceramic membrane permeate produced from PE (SE: secondary effluent. PE: primary effluent. Bar plot represent mean infectivity index)

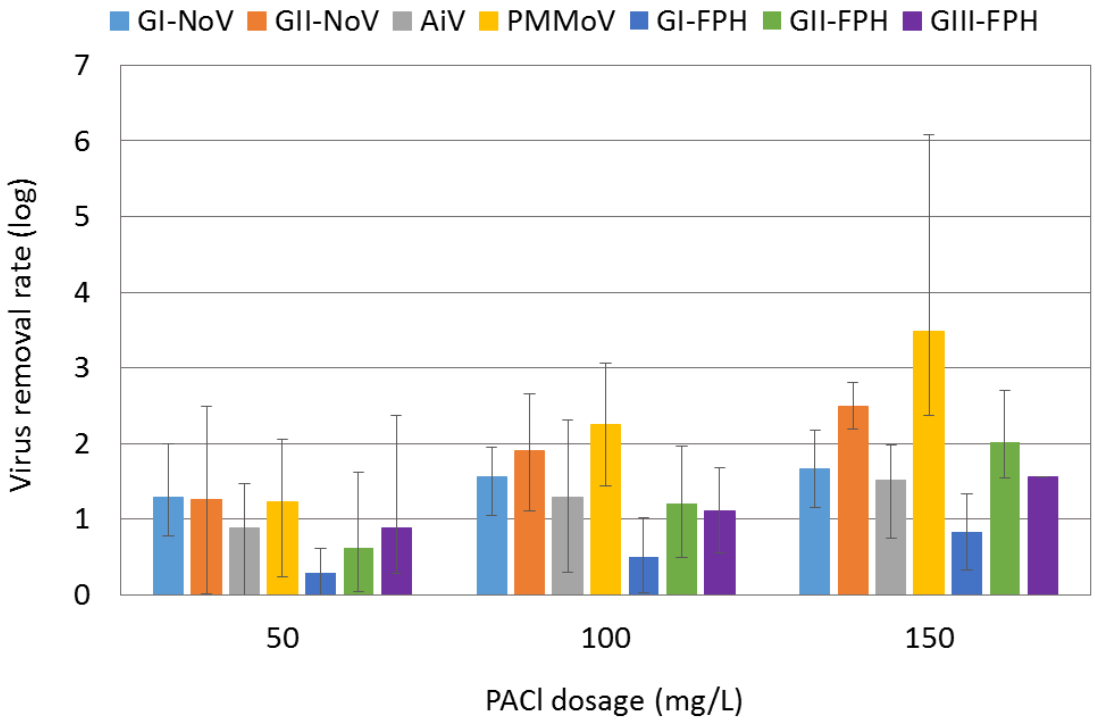

Figure S7 Removal rate of indigenous virus by coagulation and sedimentation (Source water was primary effluent. The experiment was triplicated. The value represent mean removal rate, and error bars indicate the range)

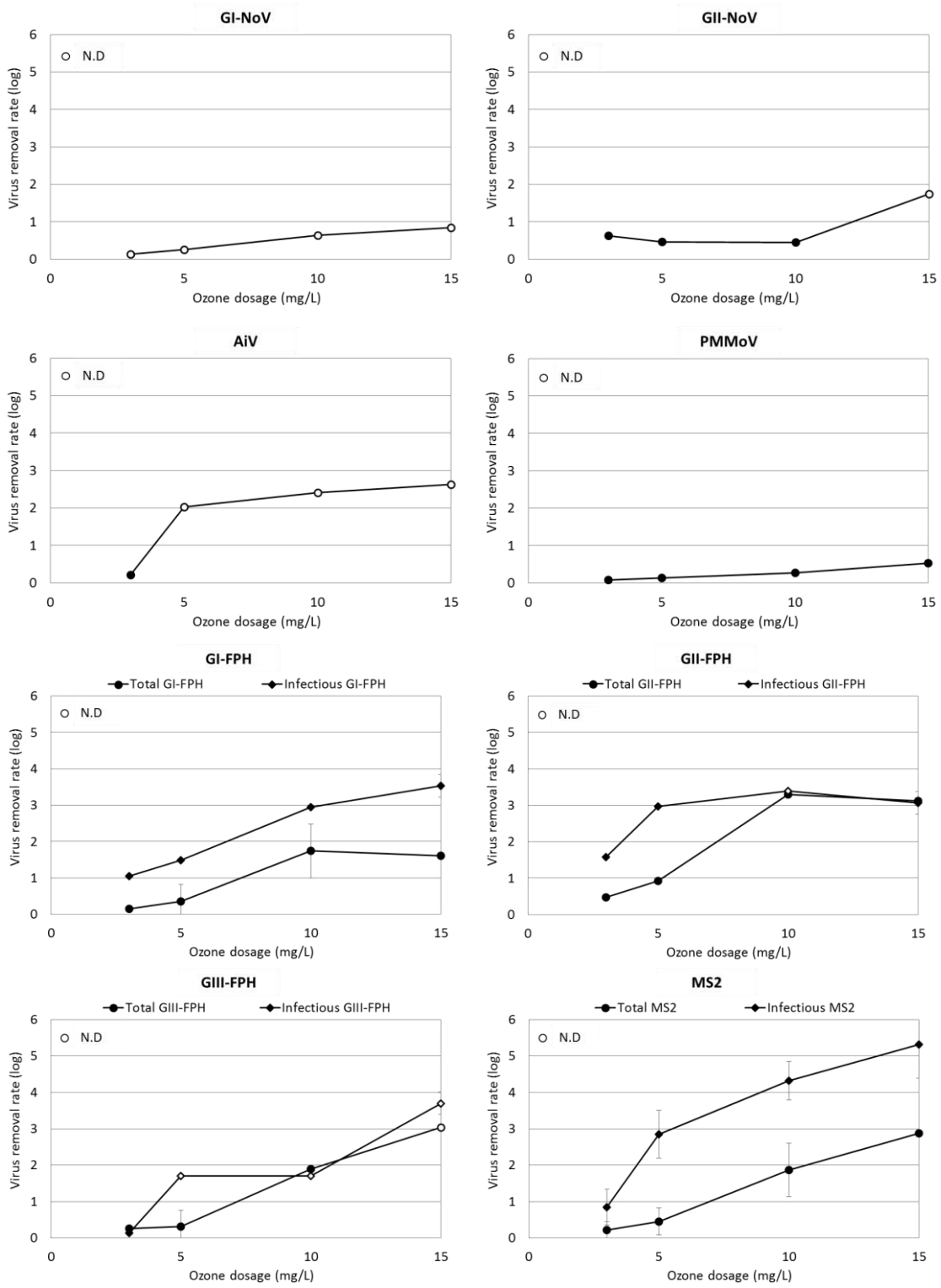

Figure S8 Mean removal rate of indigenous virus and the spiked MS2 during post-ozonation (Source water was ceramic membrane permeate produced from primary effluent. White circle or diamond indicates that virus was not detected in ozonated water. Error bar represent the standard deviation)

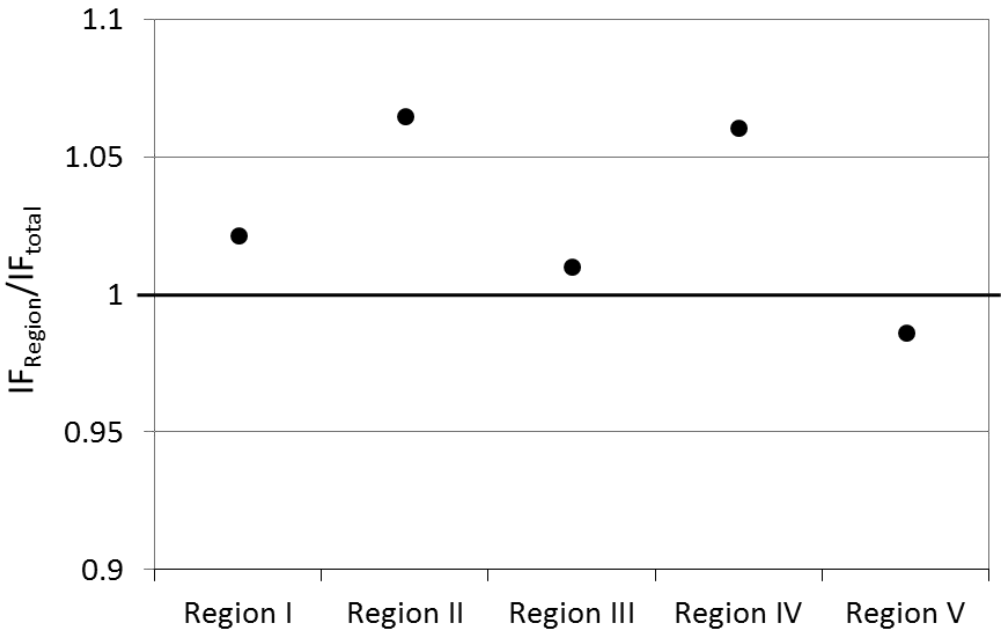

Figure S9 Comparison of $\Delta IF/IF_0$ in each region with total $\Delta IF/IF_0$ in all region ($\Delta IF/IF_0$: the relative change of cumulative fluorescence intensity. The value represent mean $\Delta IF_{Region}/IF_{total}$.)

Table S1 Energy consumption of O_3&CMF process for treating secondary effluent

O_3+PACl+CMF (kWh/m³)									
Scenario	Process 1			Process 2			Process 3		
	O_3	PACl+CMF	Total	O_3	PACl+CMF	Total	O_3	PACl+CMF	Total
Scenario 1	0.024	0.088	0.112	0.046	0.082	0.128	0.091	0.069	0.160
Scenario 2	0.024	0.076	0.100	0.046	0.070	0.116	0.091	0.060	0.151
Scenario 3	0.024	0.078	0.102	0.046	0.072	0.118	0.091	0.061	0.152
Scenario 4	0.024	0.072	0.096	0.046	0.066	0.112	0.091	0.058	0.149
Scenario 5	0.024	0.061	0.085	0.046	0.059	0.105	0.091	0	0.091
PACl+CMF+O_3 (kWh/m³)									
Scenario	Process 4			Process 5			Process 6		
	PACl+CMF	O_3	Total	PACl+CMF	O_3	Total	PACl+CMF	O_3	Total
Scenario 1	0.056	0.150	0.206	0.059	0.120	0.179	0.068	0.078	0.146
Scenario 2	0.056	0.100	0.156	0.059	0.080	0.139	0.068	0.045	0.113
Scenario 3	0.056	0.110	0.166	0.059	0.087	0.146	0.068	0.050	0.118
Scenario 4	0.056	0.089	0.145	0.059	0.070	0.129	0.068	0.034	0.102
Scenario 5	0.056	0.056	0.112	0.059	0.038	0.097	0.068	0	0.068

Table S2 Energy consumption of O_3&CMF process for treating primary effluent

O_3+PACl+CMF (kWh/m³)									
Scenario	Process 1			Process 2			Process 3		
	O_3	PACl+CMF	Total	O_3	PACl+CMF	Total	O_3	PACl+CMF	Total
Scenario 1	0.224	0.906	1.13	0.758	0.693	1.451	1.292	0.481	1.773
Scenario 2	0.224	0.704	0.928	0.758	0.491	1.249	1.292	0.279	1.571
Scenario 3	0.224	0.736	0.96	0.758	0.523	1.281	1.292	0.311	1.603
Scenario 4	0.224	0.64	0.864	0.758	0.428	1.186	1.292	0.215	1.507
Scenario 5	0.224	0.449	0.673	0.758	0.236	0.994	1.292	0	1.292
PACl+CMF+O_3 (kWh/m³)									
Scenario	Process 4			Process 5			Process 6		
	PACl+CMF	O_3	Total	PACl+CMF	O_3	Total	PACl+CMF	O_3	Total
Scenario 1	0.13	1.063	1.193	0.343	0.767	1.110	0.555	0.471	1.026
Scenario 2	0.13	0.782	0.912	0.343	0.486	0.829	0.555	0.261	0.816
Scenario 3	0.13	0.826	0.956	0.343	0.530	0.873	0.555	0.295	0.850
Scenario 4	0.13	0.693	0.823	0.343	0.422	0.765	0.555	0.191	0.746
Scenario 5	0.13	0.445	0.575	0.343	0.215	0.558	0.555	0	0.555

Table S3 The result of DALYs calculation for each exposure scenario (copies/L)

Water reclamation system		Scenario 1			Scenario 2			Scenario 3			Scenario 4			Scenario 5			Scenario 6		
		5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%
PACl+CMF (P1)		3.6×10^{-9}	1.3×10^{-7}	3.7×10^{-6}	3.7×10^{-11}	1.6×10^{-9}	5.6×10^{-8}	6.7×10^{-11}	2.9×10^{-9}	1.0×10^{-7}	8.2×10^{-12}	3.6×10^{-10}	1.2×10^{-8}	1.2×10^{-13}	4.6×10^{-12}	1.3×10^{-10}	2.6×10^{-11}	9.5×10^{-10}	2.7×10^{-8}
O_3 + PACl + CMF (P2)	P2-2	1.3×10^{-13}	3.7×10^{-12}	8.4×10^{-11}	1.5×10^{-15}	3.8×10^{-14}	1.0×10^{-12}	2.7×10^{-15}	6.9×10^{-14}	1.9×10^{-12}	3.3×10^{-16}	8.4×10^{-15}	2.3×10^{-13}	4.4×10^{-18}	1.3×10^{-16}	3.0×10^{-15}	9.2×10^{-16}	2.7×10^{-14}	6.1×10^{-13}
	P2-4	4.1×10^{-13}	1.0×10^{-11}	1.8×10^{-10}	4.9×10^{-15}	1.1×10^{-13}	2.1×10^{-12}	8.8×10^{-15}	1.9×10^{-13}	3.8×10^{-12}	1.1×10^{-15}	2.3×10^{-14}	4.6×10^{-13}	1.4×10^{-17}	3.6×10^{-16}	6.4×10^{-15}	3.0×10^{-15}	7.3×10^{-14}	1.3×10^{-12}
	P2-6	8.9×10^{-14}	3.5×10^{-12}	1.6×10^{-10}	1.2×10^{-15}	5.0×10^{-14}	2.0×10^{-12}	2.1×10^{-15}	8.9×10^{-14}	3.6×10^{-12}	2.6×10^{-16}	1.1×10^{-14}	4.4×10^{-13}	3.1×10^{-18}	1.2×10^{-16}	5.5×10^{-15}	6.5×10^{-16}	2.5×10^{-14}	1.1×10^{-12}

Table S4 The result of calculation of lifetime cancer risk caused by formaldehyde (μg/L)

Water reclamation system	Exposure route	Scenario 1			Scenario 2			Scenario 3			Scenario 4			Scenario 5			Scenario 6		
		5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%
PACl+CMF (P1)	Oral ingestion	$5.3x10^{-9}$	$1.6x10^{-8}$	$4.7x10^{-8}$	$6.1x10^{-11}$	$2.0x10^{-10}$	$5.8x10^{-10}$	$1.3x10^{-10}$	$4.0x10^{-10}$	$1.2x10^{-9}$	$1.5x10^{-11}$	$4.4x10^{-11}$	$1.3x10^{-10}$	$1.9x10^{-10}$	$5.6x10^{-10}$	$1.6x10^{-9}$	$3.4x10^{-9}$	$1.0x10^{-8}$	$3.0x10^{-8}$
	Inhalation in shower	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
	Dermal absorption	$6.1x10^{-11}$	$1.8x10^{-10}$	$5.3x10^{-10}$	-	-	-	-	-	-	-	-	-	-	-	-	$2.1x10^{-13}$	$6.3x10^{-13}$	$1.8x10^{-12}$
	Total	$5.4x10^{-9}$	$1.6x10^{-8}$	$4.7x10^{-8}$	$6.1x10^{-11}$	$2.0x10^{-10}$	$5.8x10^{-10}$	$1.3x10^{-10}$	$4.0x10^{-10}$	$1.2x10^{-9}$	$1.5x10^{-11}$	$4.4x10^{-11}$	$1.3x10^{-10}$	1.9E-10	5.6E-10	1.6E-09	$3.4x10^{-9}$	$1.0x10^{-8}$	$3.0x10^{-8}$
O_3 + PACl + CMF (P2) P2-2	Oral ingestion	$1.8x10^{-8}$	$6.3x10^{-8}$	$2.6x10^{-7}$	$2.2x10^{-10}$	$7.9x10^{-10}$	$3.2x10^{-9}$	$4.4x10^{-10}$	$1.6x10^{-9}$	$6.5x10^{-9}$	$4.9x10^{-11}$	$1.7x10^{-10}$	$7.1x10^{-10}$	$6.2x10^{-10}$	$2.2x10^{-9}$	$9.1x10^{-9}$	$1.1x10^{-8}$	$4.0x10^{-8}$	$1.6x10^{-7}$
	Inhalation in shower	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
	Dermal absorption	$2.0x10^{-10}$	$7.2x10^{-10}$	$2.9x10^{-9}$	-	-	-	-	-	-	-	-	-	-	-	-	$7.0x10^{-13}$	$2.5x10^{-12}$	$1.0x10^{-11}$
	Total	$1.8x10^{-8}$	$6.4x10^{-8}$	$2.6x10^{-7}$	$2.2x10^{-10}$	$7.9x10^{-10}$	$3.2x10^{-9}$	$4.4x10^{-10}$	$1.6x10^{-9}$	$6.5x10^{-9}$	$4.9x10^{-11}$	$1.7x10^{-10}$	$7.1x10^{-10}$	$6.2x10^{-10}$	$2.2x10^{-9}$	$9.1x10^{-9}$	$1.1x10^{-8}$	$4.0x10^{-8}$	$1.6x10^{-7}$
P2-4	Oral ingestion	$3.5x10^{-8}$	$1.1x10^{-7}$	$3.3x10^{-7}$	$4.4x10^{-10}$	$1.4x10^{-9}$	$4.1x10^{-9}$	$8.9x10^{-10}$	$2.7x10^{-9}$	$8.1x10^{-9}$	$9.8x10^{-11}$	$3.0x10^{-10}$	$9.0x10^{-10}$	$1.2x10^{-9}$	$3.8x10^{-9}$	$1.1x10^{-8}$	$2.3x10^{-8}$	$6.9x10^{-8}$	$2.1x10^{-7}$
	Inhalation in shower	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
	Dermal absorption	$4.0x10^{-10}$	$1.2x10^{-9}$	$3.7x10^{-9}$	-	-	-	-	-	-	-	-	-	-	-	-	$1.4x10^{-12}$	$4.3x10^{-12}$	$1.3x10^{-11}$
	Total	$3.6x10^{-8}$	$1.1x10^{-7}$	$3.3x10^{-7}$	$4.4x10^{-10}$	$1.4x10^{-9}$	$4.1x10^{-9}$	$8.9x10^{-10}$	$2.7x10^{-9}$	$8.1x10^{-9}$	$9.8x10^{-11}$	$3.0x10^{-10}$	$9.0x10^{-10}$	$1.2x10^{-9}$	$3.8x10^{-9}$	$1.1x10^{-8}$	$2.3x10^{-8}$	$6.9x10^{-8}$	$2.1x10^{-7}$
P2-6	Oral ingestion	$4.0x10^{-8}$	$1.4x10^{-7}$	$5.3x10^{-7}$	$5.0x10^{-10}$	$1.8x10^{-9}$	$6.6x10^{-9}$	$1.0x10^{-10}$	$3.6x10^{-9}$	$1.3x10^{-9}$	$1.1x10^{-10}$	$4.0x10^{-10}$	$1.5x10^{-9}$	$1.4x10^{-9}$	$5.0x10^{-9}$	$1.9x10^{-8}$	$2.5x10^{-8}$	$9.1x10^{-8}$	$3.4x10^{-7}$
	Inhalation in shower	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
	Dermal absorption	$4.5x10^{-10}$	$1.6x10^{-9}$	$6.0x10^{-9}$	-	-	-	-	-	-	-	-	-	-	-	-	$1.6x10^{-12}$	$5.7x10^{-12}$	$2.1x10^{-11}$
	Total	$4.0x10^{-8}$	$1.5x10^{-7}$	$5.4x10^{-7}$	$5.0x10^{-10}$	$1.8x10^{-9}$	$6.6x10^{-9}$	$1.0x10^{-10}$	$3.6x10^{-9}$	$1.3x10^{-9}$	$1.1x10^{-10}$	$4.0x10^{-10}$	$1.5x10^{-9}$	$1.4x10^{-9}$	$5.0x10^{-9}$	$1.9x10^{-8}$	$2.5x10^{-8}$	$9.1x10^{-8}$	$3.4x10^{-7}$
O_3+BAC+PACl+CMF (P3)	Oral ingestion	$3.8x10^{-9}$	$2.5x10^{-8}$	$1.1x10^{-7}$	$4.8x10^{-11}$	$3.1x10^{-10}$	$1.3x10^{-9}$	$8.9x10^{-10}$	$2.7x10^{-9}$	$8.1x10^{-9}$	$1.1x10^{-11}$	$6.9x10^{-11}$	$2.9x10^{-10}$	$1.3x10^{-10}$	$8.7x10^{-10}$	$3.7x10^{-9}$	$2.4x10^{-9}$	$1.6x10^{-8}$	$6.8x10^{-8}$
	Inhalation in shower	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
	Dermal absorption	$4.3x10^{-11}$	$2.8x10^{-10}$	$1.2x10^{-9}$	-	-	-	-	-	-	-	-	-	-	-	-	$1.5x10^{-13}$	$9.8x10^{-13}$	$4.2x10^{-12}$
	Total	$3.9x10^{-9}$	$2.5x10^{-8}$	$1.1x10^{-7}$	$4.8x10^{-11}$	$3.1x10^{-10}$	$1.3x10^{-9}$	$8.9x10^{-10}$	$2.7x10^{-9}$	$8.1x10^{-9}$	$1.1x10^{-11}$	$6.9x10^{-11}$	$2.9x10^{-10}$	$1.3x10^{-10}$	$8.7x10^{-10}$	$3.7x10^{-9}$	$2.4x10^{-9}$	$1.6x10^{-8}$	$6.8x10^{-8}$

Table S5 The result of calculation of lifetime cancer risk caused by *N*-nitrosodimethylamine (ng/L)

Water reclamation system	Exposure route	Scenario 1			Scenario 2			Scenario 3			Scenario 4			Scenario 5			Scenario 6		
		5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%
PACl+CMF (P1)	Oral ingestion	4.3×10^{-8}	7.5×10^{-8}	1.4×10^{-7}	5.4×10^{-10}	9.4×10^{-10}	1.8×10^{-9}	1.1×10^{-9}	1.9×10^{-9}	3.6×10^{-9}	1.2×10^{-10}	2.1×10^{-10}	4.0×10^{-10}	1.5×10^{-9}	2.6×10^{-9}	5.0×10^{-9}	3.8×10^{-6}	6.4×10^{-6}	1.2×10^{-5}
	Inhalation in shower	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
	Dermal absorption	1.5×10^{-10}	2.7×10^{-10}	5.1×10^{-10}	-	-	-	-	-	-	-	-	-	-	-	-	7.3×10^{-11}	1.2×10^{-10}	2.2×10^{-10}
	Total	4.3×10^{-8}	7.6×10^{-8}	1.4×10^{-7}	5.4×10^{-10}	9.4×10^{-10}	1.8×10^{-9}	1.1×10^{-9}	1.9×10^{-9}	3.6×10^{-9}	1.2×10^{-10}	2.1×10^{-10}	4.0×10^{-10}	1.5×10^{-9}	2.6×10^{-9}	5.0×10^{-9}	3.8×10^{-6}	6.4×10^{-6}	1.2×10^{-5}
O_3 + PACl + CMF (P2) P2-2	Oral ingestion	1.9×10^{-8}	6.3×10^{-8}	2.9×10^{-7}	2.3×10^{-10}	7.8×10^{-10}	3.6×10^{-9}	4.7×10^{-10}	1.6×10^{-9}	7.2×10^{-9}	5.1×10^{-11}	1.7×10^{-10}	7.9×10^{-10}	6.5×10^{-10}	2.2×10^{-9}	1.8×10^{-8}	1.4×10^{-6}	4.1×10^{-6}	1.7×10^{-5}
	Inhalation in shower	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
	Dermal absorption	6.6×10^{-11}	2.2×10^{-10}	1.0×10^{-9}	-	-	-	-	-	-	-	-	-	-	-	-	2.6×10^{-11}	8.0×10^{-11}	3.3×10^{-10}
	Total	1.9×10^{-8}	6.3×10^{-8}	2.9×10^{-7}	2.3×10^{-10}	7.8×10^{-10}	3.6×10^{-9}	4.7×10^{-10}	1.6×10^{-9}	7.2×10^{-9}	5.1×10^{-11}	1.7×10^{-10}	7.9×10^{-10}	6.5×10^{-10}	2.2×10^{-9}	1.8×10^{-8}	1.4×10^{-6}	4.1×10^{-6}	1.7×10^{-5}
P2-4	Oral ingestion	1.4×10^{-8}	6.5×10^{-8}	2.2×10^{-7}	1.7×10^{-10}	8.2×10^{-10}	2.7×10^{-9}	3.5×10^{-10}	1.6×10^{-9}	5.5×10^{-9}	3.8×10^{-11}	1.8×10^{-10}	6.0×10^{-10}	4.9×10^{-10}	2.3×10^{-9}	7.7×10^{-9}	9.7×10^{-7}	4.3×10^{-6}	1.3×10^{-5}
	Inhalation in shower	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
	Dermal absorption	4.9×10^{-11}	2.3×10^{-10}	7.8×10^{-10}	-	-	-	-	-	-	-	-	-	-	-	-	1.9×10^{-11}	8.4×10^{-11}	2.6×10^{-10}
	Total	1.4×10^{-8}	6.6×10^{-8}	2.2×10^{-7}	2.3×10^{-10}	7.8×10^{-10}	3.6×10^{-9}	4.7×10^{-10}	1.6×10^{-9}	7.2×10^{-9}	5.1×10^{-11}	1.7×10^{-10}	7.9×10^{-10}	6.5×10^{-10}	2.2×10^{-9}	1.8×10^{-8}	9.7×10^{-7}	4.3×10^{-6}	1.3×10^{-5}
P2-6	Oral ingestion	1.6×10^{-8}	7.2×10^{-8}	2.2×10^{-7}	2.0×10^{-10}	9.0×10^{-10}	2.7×10^{-9}	4.0×10^{-10}	1.8×10^{-9}	5.4×10^{-9}	4.4×10^{-11}	2.0×10^{-10}	6.0×10^{-10}	5.6×10^{-10}	2.5×10^{-9}	7.6×10^{-9}	9.7×10^{-7}	5.4×10^{-6}	1.5×10^{-5}
	Inhalation in shower	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
	Dermal absorption	5.7×10^{-11}	2.6×10^{-10}	7.8×10^{-10}	-	-	-	-	-	-	-	-	-	-	-	-	1.9×10^{-11}	1.1×10^{-10}	2.9×10^{-10}
	Total	1.6×10^{-8}	7.2×10^{-8}	2.2×10^{-7}	2.3×10^{-10}	7.8×10^{-10}	3.6×10^{-9}	4.7×10^{-10}	1.6×10^{-9}	7.2×10^{-9}	5.1×10^{-11}	1.7×10^{-10}	7.9×10^{-10}	6.5×10^{-10}	2.2×10^{-9}	1.8×10^{-8}	9.7×10^{-7}	5.4×10^{-6}	1.5×10^{-5}
O_3+BAC+PACl+CMF (P3)	Oral ingestion	2.0×10^{-9}	2.3×10^{-8}	4.7×10^{-8}	2.4×10^{-11}	2.9×10^{-10}	5.9×10^{-10}	4.9×10^{-11}	5.7×10^{-10}	1.2×10^{-9}	5.4×10^{-12}	6.3×10^{-11}	1.3×10^{-10}	6.8×10^{-11}	8.0×10^{-10}	1.7×10^{-9}	9.3×10^{-8}	2.0×10^{-6}	4.5×10^{-6}
	Inhalation in shower	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
	Dermal absorption	6.9×10^{-12}	8.2×10^{-11}	1.7×10^{-10}	-	-	-	-	-	-	-	-	-	-	-	-	1.8×10^{-12}	4.0×10^{-11}	8.7×10^{-11}
	Total	2.0×10^{-9}	2.3×10^{-8}	4.7×10^{-8}	2.4×10^{-11}	2.9×10^{-10}	5.9×10^{-10}	4.9×10^{-11}	5.7×10^{-10}	1.2×10^{-9}	5.4×10^{-12}	6.3×10^{-11}	1.3×10^{-10}	6.8×10^{-11}	8.0×10^{-10}	1.7×10^{-9}	9.3×10^{-8}	2.0×10^{-6}	4.5×10^{-6}

Table S6 The result of calculation of lifetime cancer risk caused by chloroform (μg/L)

Water reclamation system	Exposure route	Scenario 1			Scenario 2			Scenario 3			Scenario 4			Scenario 5			Scenario 6		
		5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%
PACl+CMF (P1)	Oral ingestion	8.8×10^{-9}	1.3×10^{-8}	2.0×10^{-8}	1.1×10^{-10}	1.7×10^{-10}	2.5×10^{-10}	2.2×10^{-10}	3.3×10^{-10}	5.0×10^{-10}	2.4×10^{-11}	3.7×10^{-11}	5.5×10^{-11}	3.1×10^{-10}	4.7×10^{-10}	7.0×10^{-10}	7.3×10^{-7}	1.1×10^{-6}	1.7×10^{-6}
	Inhalation in shower	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
	Dermal absorption	6.1×10^{-11}	9.2×10^{-11}	1.4×10^{-10}	-	-	-	-	-	-	-	-	-	-	-	-	2.8×10^{-11}	4.2×10^{-11}	6.4×10^{-11}
	Total	8.8×10^{-9}	1.3×10^{-8}	2.0×10^{-8}	1.1×10^{-10}	1.7×10^{-10}	2.5×10^{-10}	2.2×10^{-10}	3.3×10^{-10}	5.0×10^{-10}	2.4×10^{-11}	3.7×10^{-11}	5.5×10^{-11}	3.1×10^{-10}	4.7×10^{-10}	7.0×10^{-10}	7.3×10^{-7}	1.1×10^{-6}	1.7×10^{-6}
O_3 + PACl + CMF (P2) P2-2	Oral ingestion	5.5×10^{-9}	9.1×10^{-9}	2.0×10^{-8}	6.9×10^{-11}	1.1×10^{-10}	2.4×10^{-10}	1.4×10^{-10}	2.3×10^{-10}	4.9×10^{-10}	1.5×10^{-11}	2.5×10^{-11}	5.4×10^{-11}	1.9×10^{-10}	3.2×10^{-10}	6.9×10^{-10}	3.6×10^{-7}	7.0×10^{-7}	1.2×10^{-6}
	Inhalation in shower	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
	Dermal absorption	6.3×10^{-11}	1.0×10^{-10}	2.2×10^{-10}	-	-	-	-	-	-	-	-	-	-	-	-	2.2×10^{-11}	4.4×10^{-11}	7.2×10^{-11}
	Total	5.6×10^{-9}	9.2×10^{-9}	2.0×10^{-8}	6.9×10^{-11}	1.1×10^{-10}	2.4×10^{-10}	1.4×10^{-10}	2.3×10^{-10}	4.9×10^{-10}	1.5×10^{-11}	2.5×10^{-11}	5.4×10^{-11}	1.9×10^{-10}	3.2×10^{-10}	6.9×10^{-10}	3.6×10^{-7}	7.0×10^{-7}	1.2×10^{-6}
P2-4	Oral ingestion	2.4×10^{-9}	9.5×10^{-9}	1.5×10^{-8}	3.0×10^{-11}	1.2×10^{-10}	1.9×10^{-10}	5.9×10^{-11}	2.4×10^{-10}	9.9×10^{-10}	6.5×10^{-12}	2.6×10^{-11}	4.3×10^{-11}	8.3×10^{-11}	3.3×10^{-10}	5.4×10^{-10}	1.4×10^{-7}	7.4×10^{-7}	1.2×10^{-6}
	Inhalation in shower	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
	Dermal absorption	2.7×10^{-11}	1.1×10^{-10}	1.8×10^{-10}	-	-	-	-	-	-	-	-	-	-	-	-	8.8×10^{-12}	4.6×10^{-11}	7.7×10^{-11}
	Total	2.4×10^{-9}	9.6×10^{-9}	1.6×10^{-8}	3.0×10^{-11}	1.2×10^{-10}	1.9×10^{-10}	5.9×10^{-11}	2.4×10^{-10}	9.9×10^{-10}	6.5×10^{-12}	2.6×10^{-11}	4.3×10^{-11}	8.3×10^{-11}	3.3×10^{-10}	5.4×10^{-10}	1.4×10^{-7}	7.4×10^{-7}	1.2×10^{-6}
P2-6	Oral ingestion	8.9×10^{-10}	1.1×10^{-8}	2.5×10^{-8}	1.1×10^{-11}	1.3×10^{-10}	3.2×10^{-10}	2.2×10^{-11}	2.6×10^{-10}	6.3×10^{-10}	2.5×10^{-12}	2.9×10^{-11}	6.9×10^{-11}	3.1×10^{-11}	3.7×10^{-10}	8.8×10^{-10}	5.3×10^{-8}	8.1×10^{-7}	1.4×10^{-6}
	Inhalation in shower	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
	Dermal absorption	1.0×10^{-11}	1.2×10^{-10}	2.9×10^{-10}	-	-	-	-	-	-	-	-	-	-	-	-	3.3×10^{-12}	5.1×10^{-11}	8.6×10^{-11}
	Total	9.0×10^{-10}	1.1×10^{-8}	2.5×10^{-8}	1.1×10^{-11}	1.3×10^{-10}	3.2×10^{-10}	2.2×10^{-11}	2.6×10^{-10}	6.3×10^{-10}	2.5×10^{-12}	2.9×10^{-11}	6.9×10^{-11}	3.1×10^{-11}	3.7×10^{-10}	8.8×10^{-10}	5.3×10^{-8}	8.1×10^{-7}	1.4×10^{-6}
O_3+BAC+PACl+CMF (P3)	Oral ingestion	4.5×10^{-10}	8.2×10^{-9}	1.4×10^{-8}	5.6×10^{-12}	1.0×10^{-10}	1.7×10^{-10}	5.9×10^{-11}	2.4×10^{-10}	3.9×10^{-10}	1.2×10^{-12}	2.3×10^{-11}	3.8×10^{-11}	1.6×10^{-11}	2.9×10^{-10}	4.9×10^{-10}	4.7×10^{-9}	4.3×10^{-7}	8.0×10^{-7}
	Inhalation in shower	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
	Dermal absorption	3.1×10^{-12}	5.7×10^{-11}	9.7×10^{-11}	-	-	-	-	-	-	-	-	-	-	-	-	1.8×10^{-13}	1.6×10^{-11}	3.0×10^{-11}
	Total	4.5×10^{-10}	8.3×10^{-9}	1.4×10^{-8}	5.6×10^{-12}	1.0×10^{-10}	1.7×10^{-10}	5.9×10^{-11}	2.4×10^{-10}	3.9×10^{-10}	1.2×10^{-12}	2.3×10^{-11}	3.8×10^{-11}	1.6×10^{-11}	2.9×10^{-10}	4.9×10^{-10}	4.7×10^{-9}	4.3×10^{-7}	8.0×10^{-7}